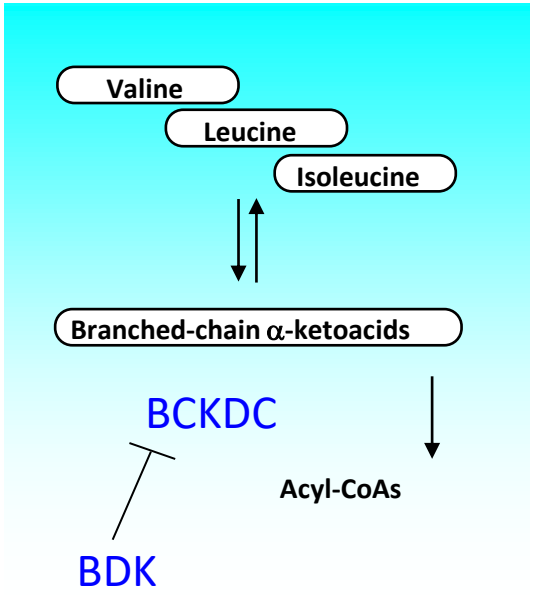
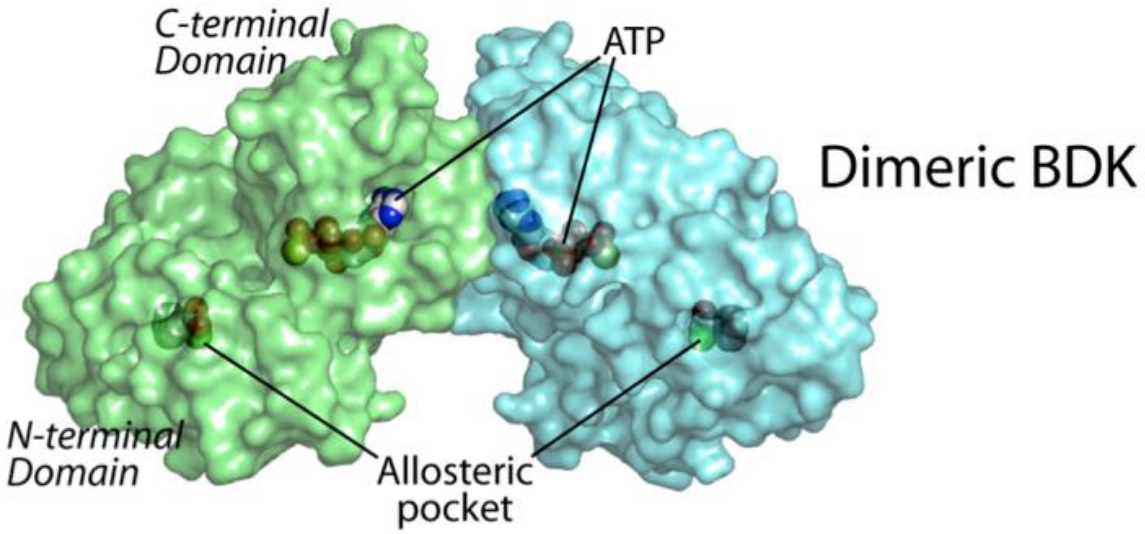
Drug Discovery for Maple Syrup Urine Disease

Disease and Target

Structure and Mechanism

Compounds and Inhibition





Max Wynn

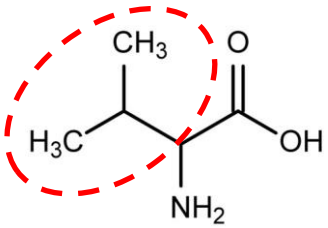
June 28, 2024

THE MILLION DOLLAR BIKE RIDE
FOR RARE DISEASES

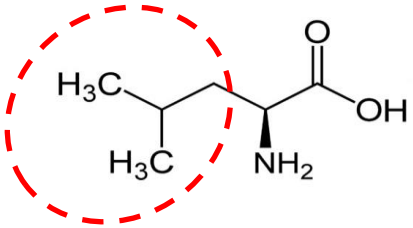
The University of Pennsylvania's Dr. Jim Wilson turned his love of cycling into an annual event that has channeled $17 million into rare disease research.

What are branched-chain amino acids?

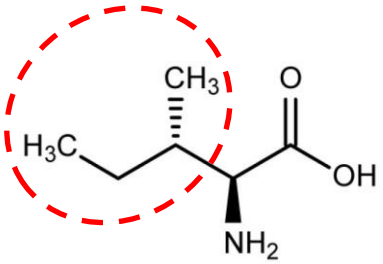
- So named due to the presence of branched aliphatic sidechain in chemical structures
- Consist of 35% of essential amino acids in muscle protein
- Comprise 50% of essential amino acids in dietary intake
- Required for protein and lipid synthesis as well as metabolic signaling and transcriptional control



Valine

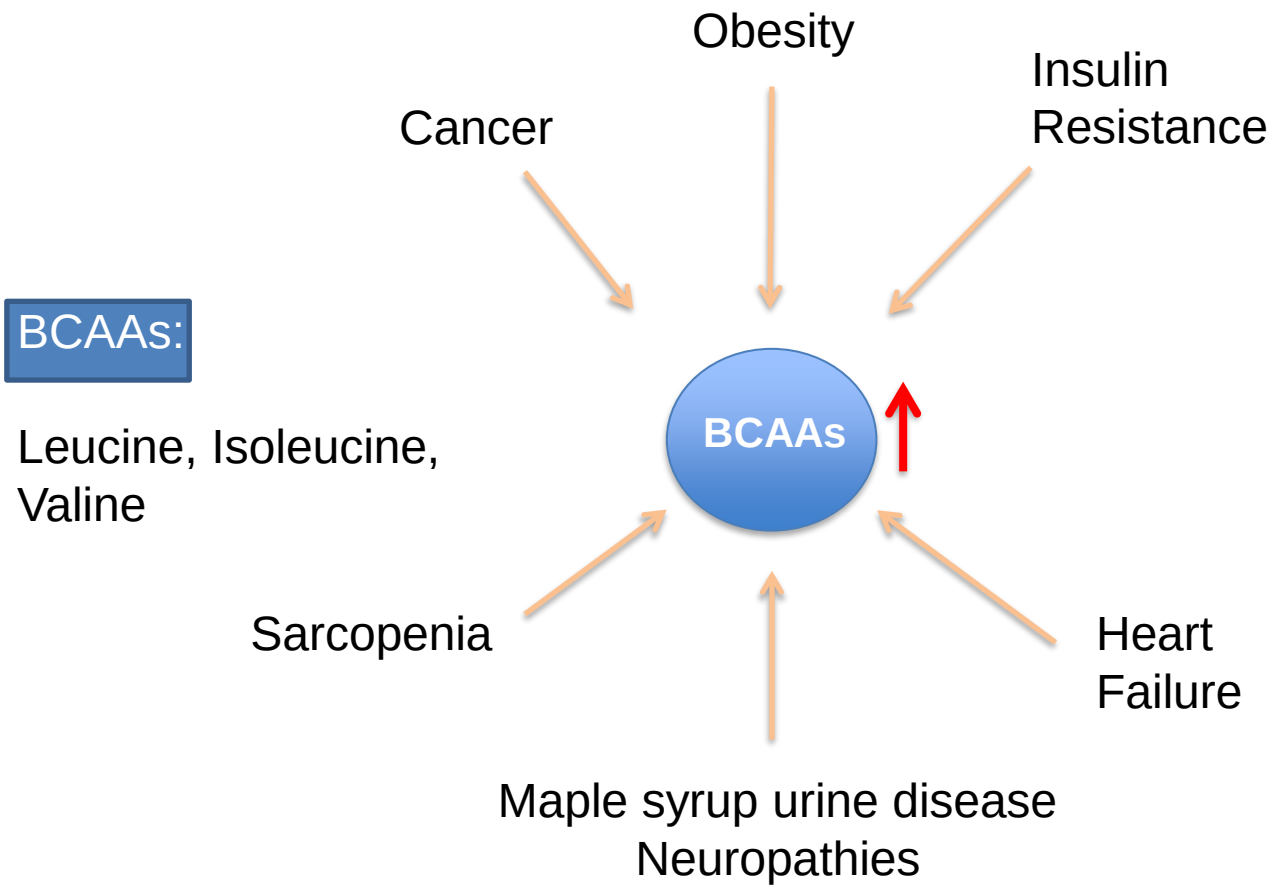


Leucine

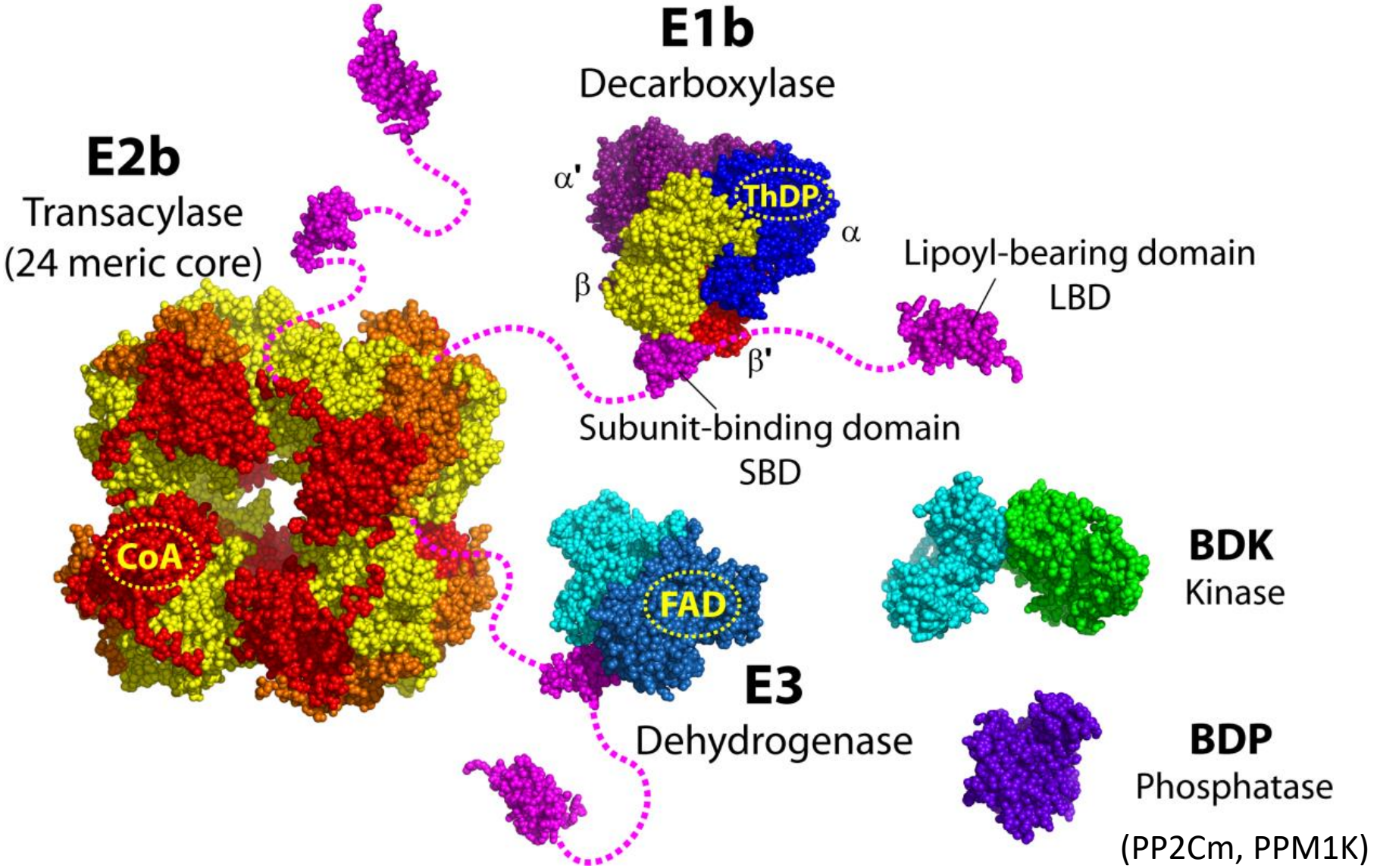


Isoleucine

Elevated BCAAs are both biomarkers and causal factors in multiple human diseases

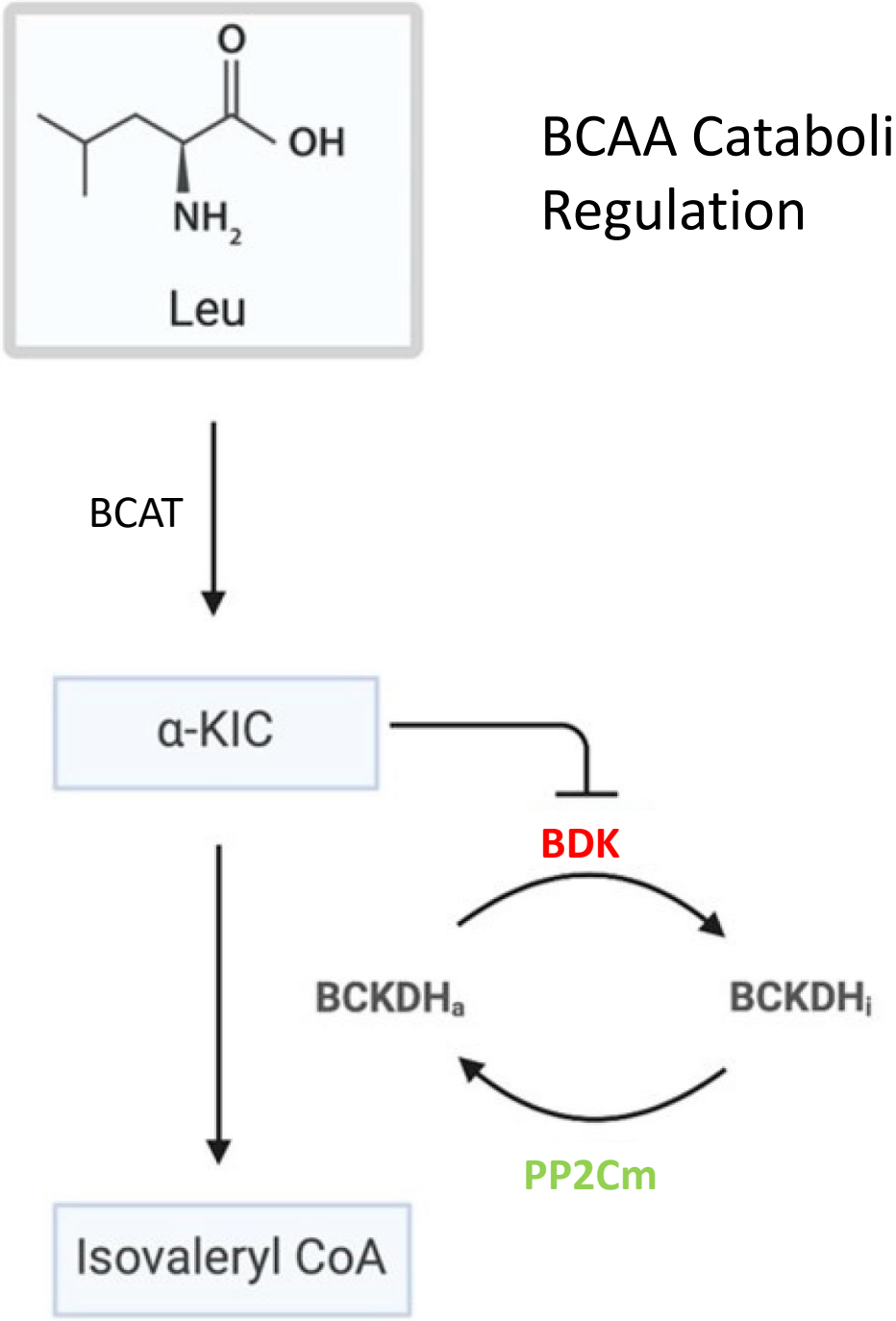


BCKDC, a 4.5 MDa machine

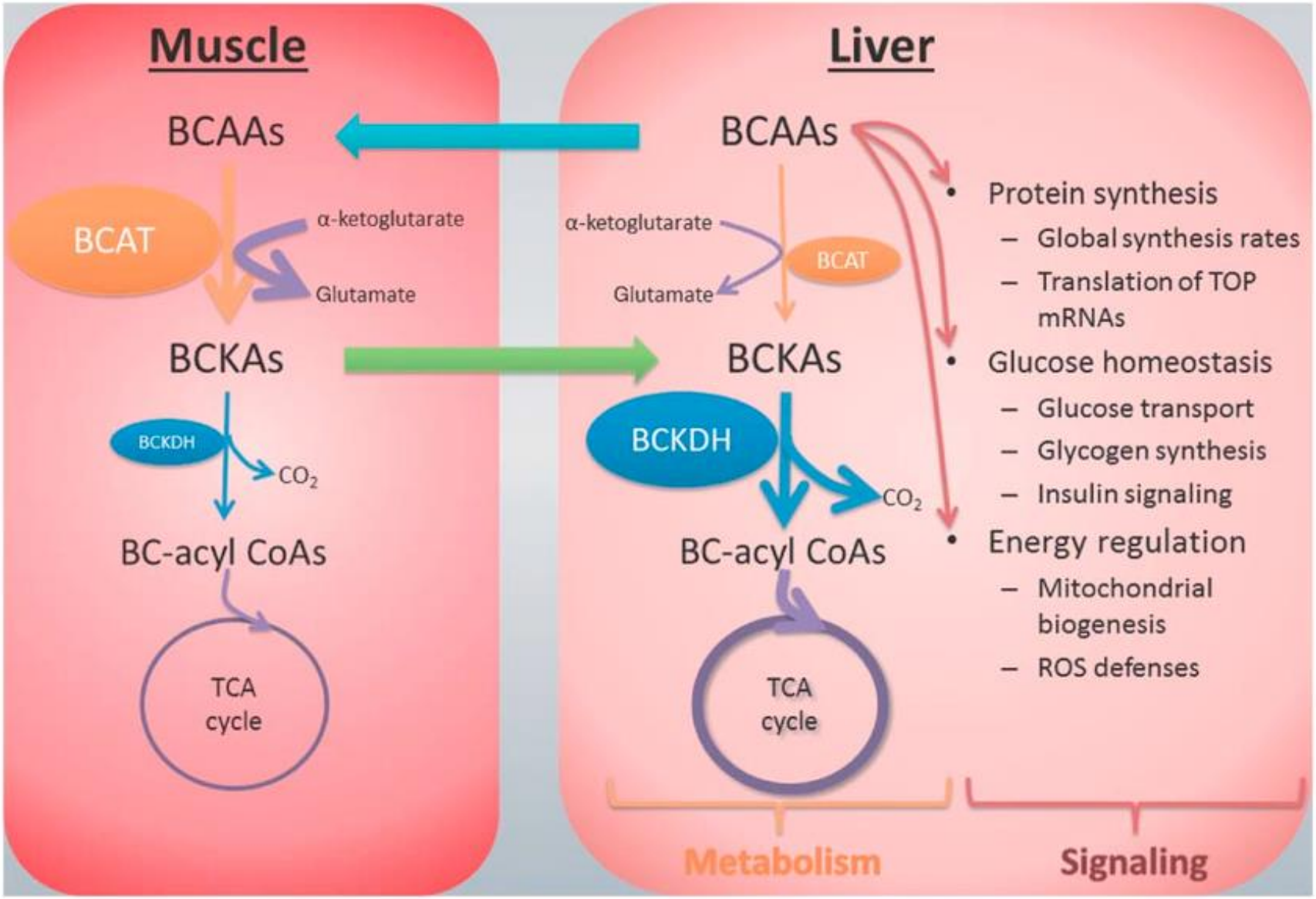


$R\text{-}COCOOH + NAD^+ + CoA \rightarrow R\text{-}CO\text{-}CoA + CO_2 + NADH_2^+$

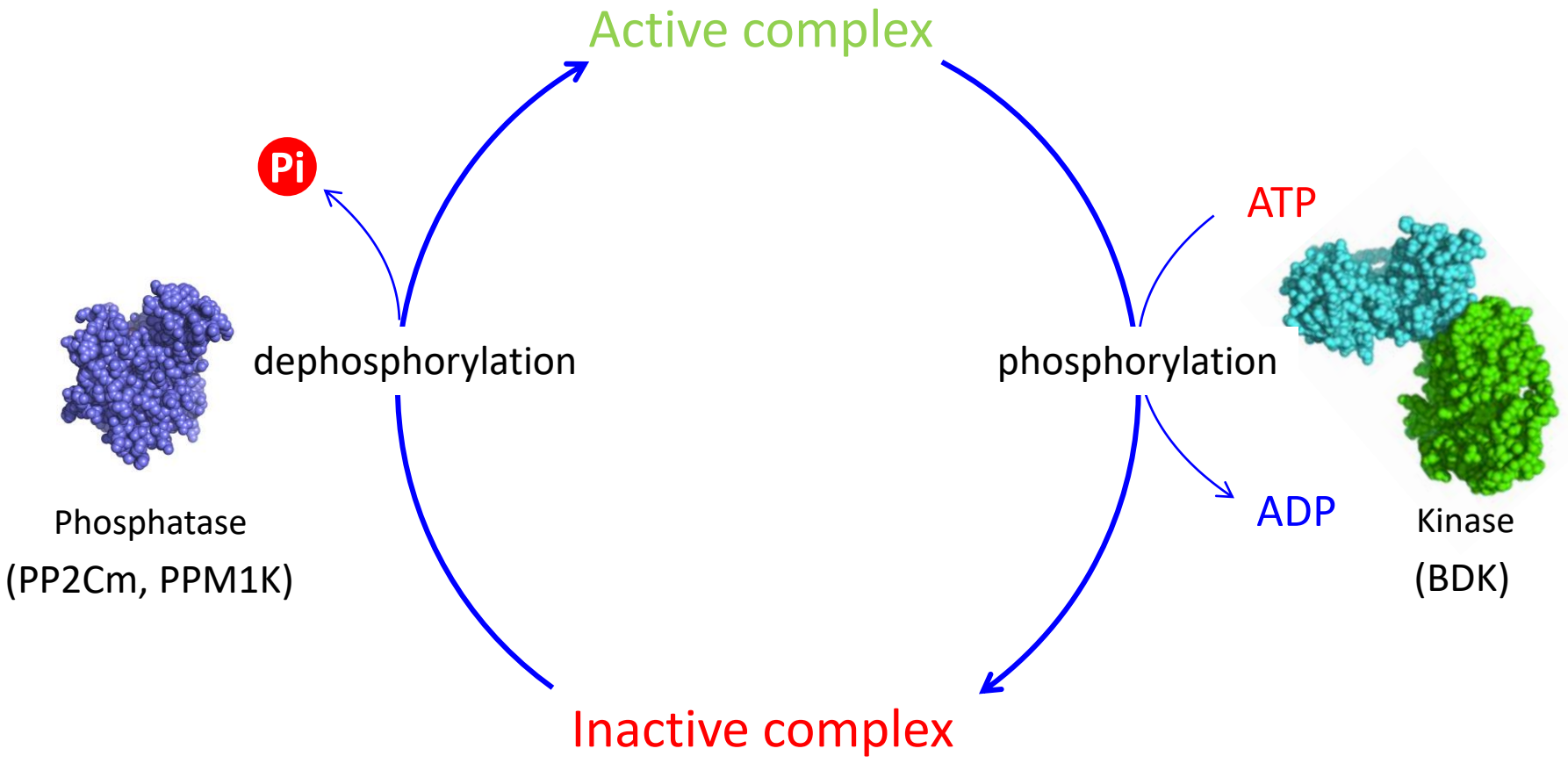
BCAA Catabolic Pathway and Regulation



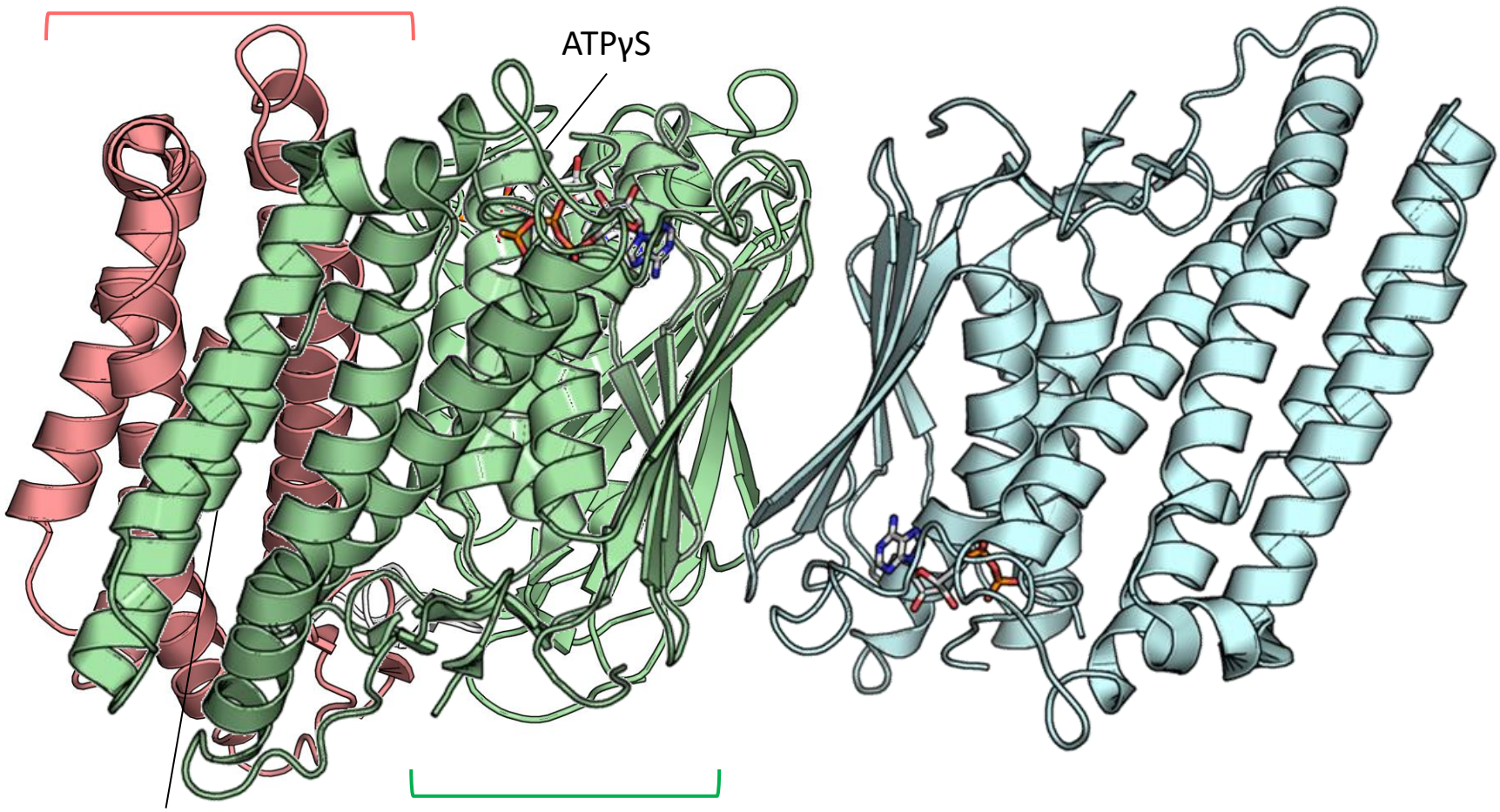
Inter-organ crosstalk between liver and muscle in BCAA degradation



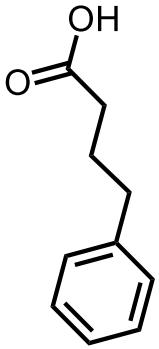
Complex activity is regulated by a reversible phosphorylation cycle



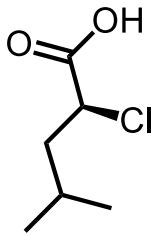
BDK is a homodimer



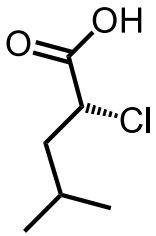
S-CPP is synthesized from PB and *(S)*-CIC



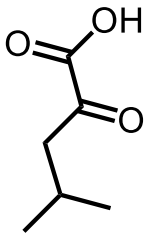
Phenylbutyrate



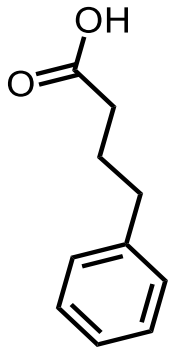
(S)-Chloroisocaproate



(R)-Chloroisocaproate

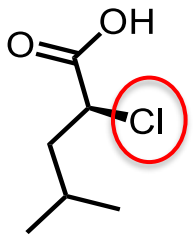


Ketoisocaproate (KIC)

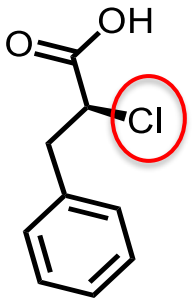


Phenylbutyrate

+



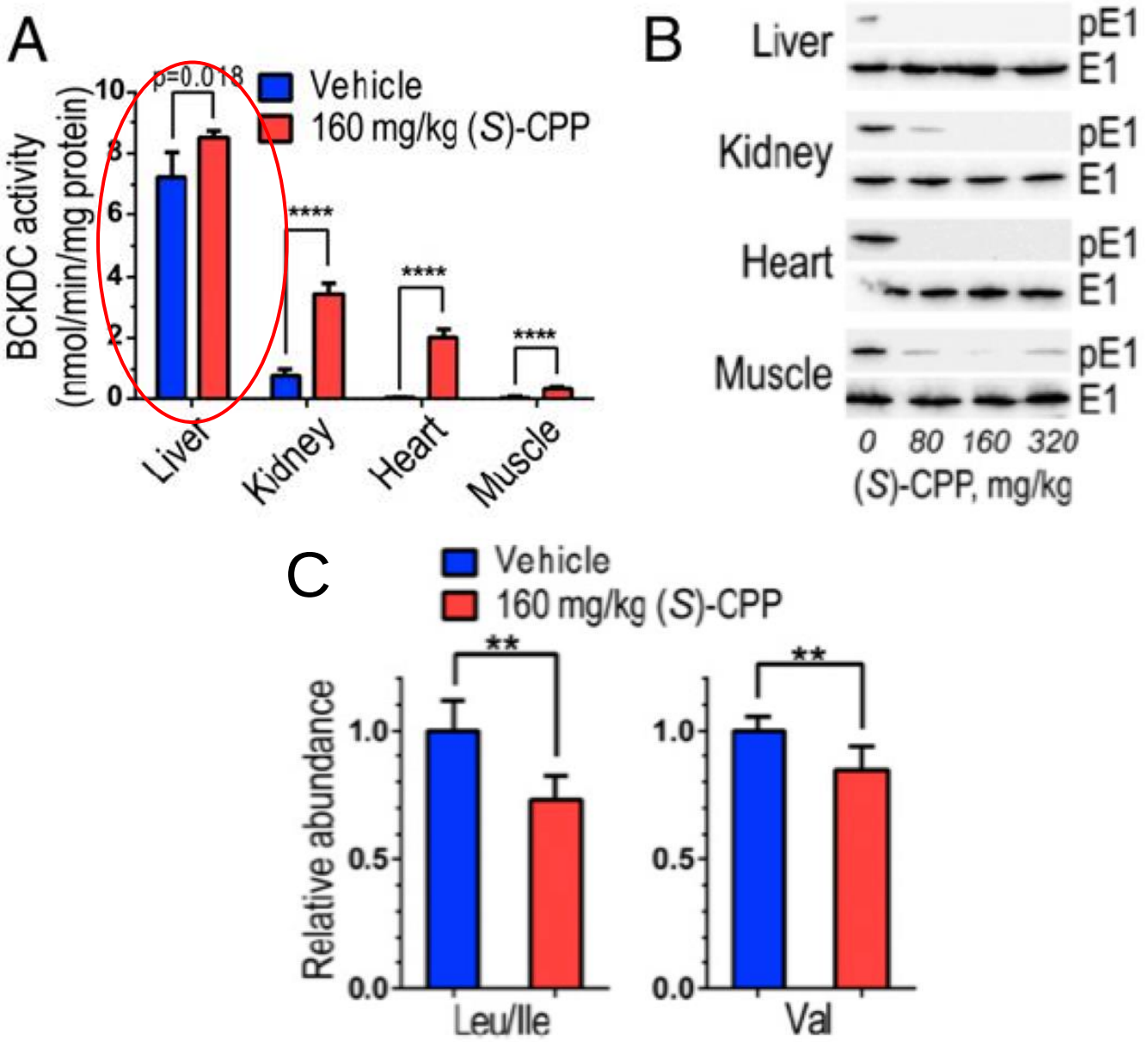
(S)-Chloroisocaproate



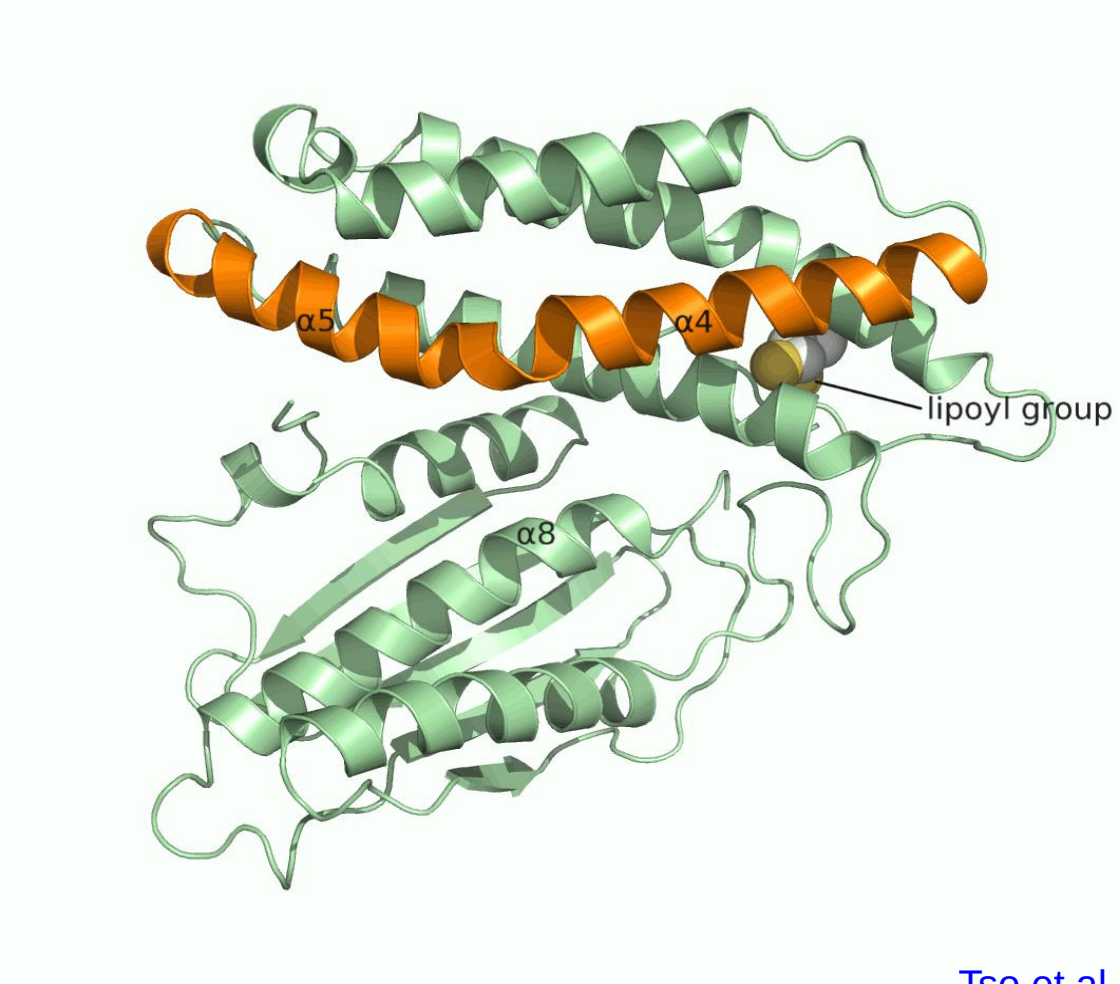
(S)-α-cholorophenylproprionate (S-CPP)

Uttam Tambar, Xiangbing (Ben) Qi and Scott Tso

(S)-CPP is effective in enhancing BDKDC activity and BCAA oxidation in wild-type mice

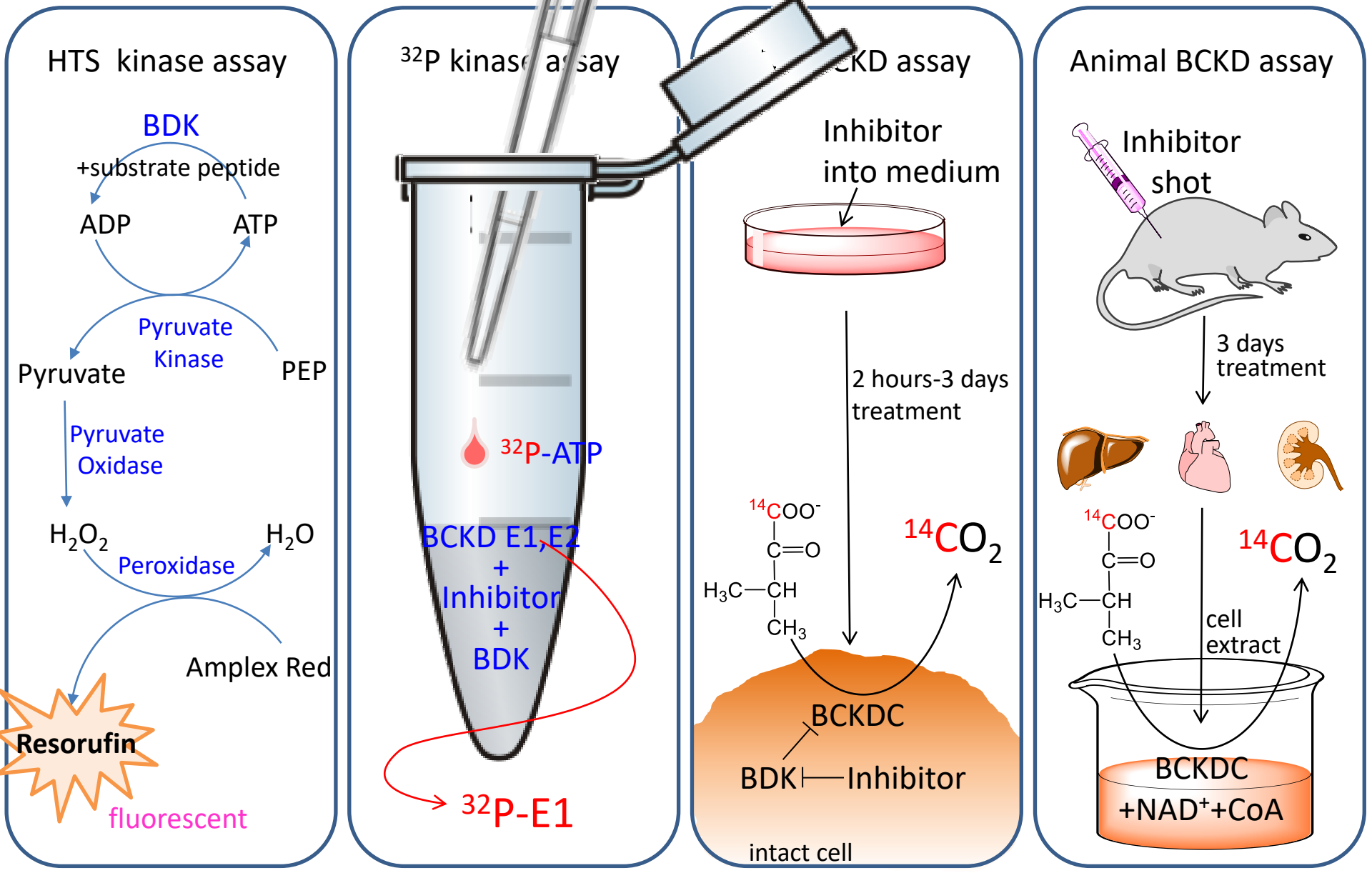


Movements of a long helical rod induced by bound (*S*)-CPP

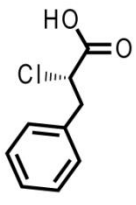


Tso et al. PNAS, 2013

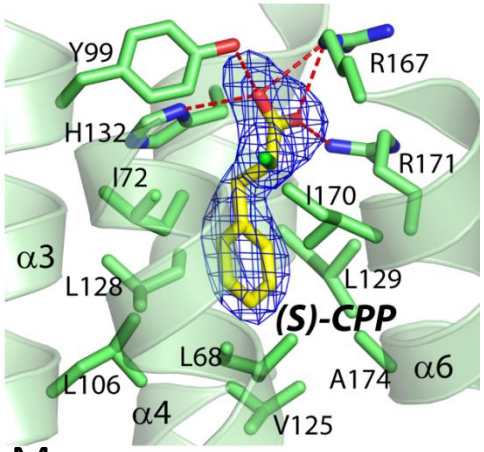
The Assays


HTS kinase assay
BDK
+substrate peptide
ADP
ATP
Pyruvate
Kinase
Pyruvate
PEP
Pyruvate
Oxidase
H_2O_2
H_2O
Peroxidase
Amplex Red
Resorufin
fluorescent
^{32}P kinase assay
^{32}P-ATP
BCKD E1, E2
+
Inhibitor
+
BDK
^{32}P-E1
CKD assay
Inhibitor
into medium
2 hours-3 days
treatment
$^{14}COO^-$
C=O
H_3C—CH
CH_3
$^{14}CO_2$
BCKDC
BDK⊣Inhibitor
intact cell
Animal BCKD assay
Inhibitor
shot
3 days
treatment
$^{14}COO^-$
C=O
H_3C—CH
CH_3
$^{14}CO_2$
cell
extract
BCKDC
+NAD$^+$+CoA

Novel allosteric inhibitors for mitochondrial BCKD kinase

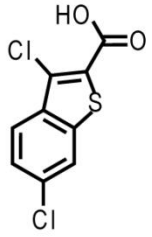


S-CPP

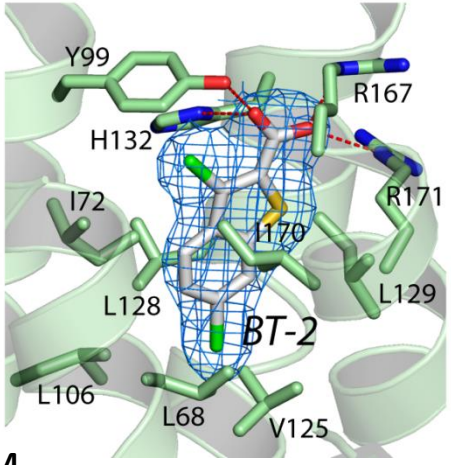


IC_{50} = 6.3 µM

Tso *et al.* 2013, *PNAS* **110**, 9728



BT2

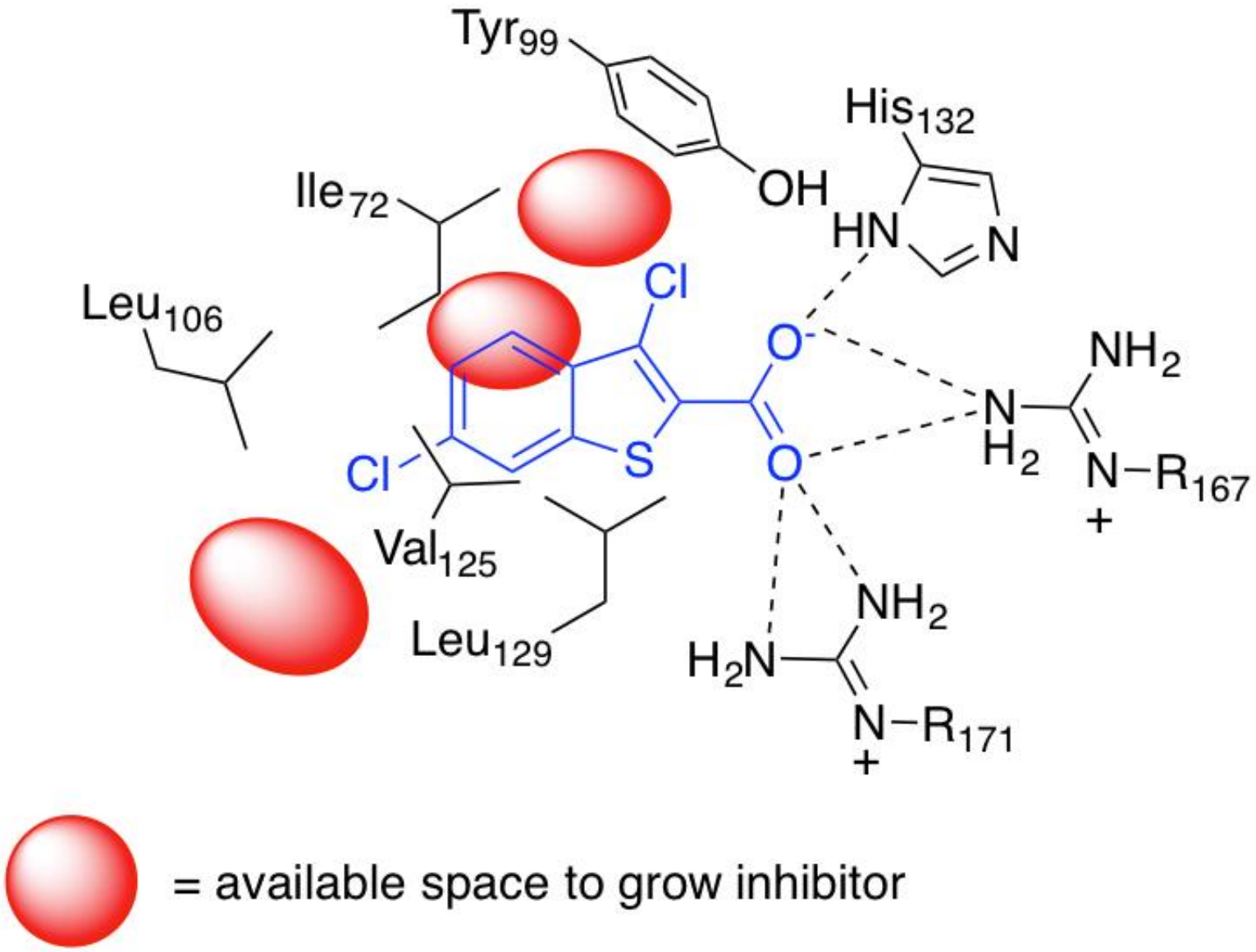


IC_{50} = 3.2 µM

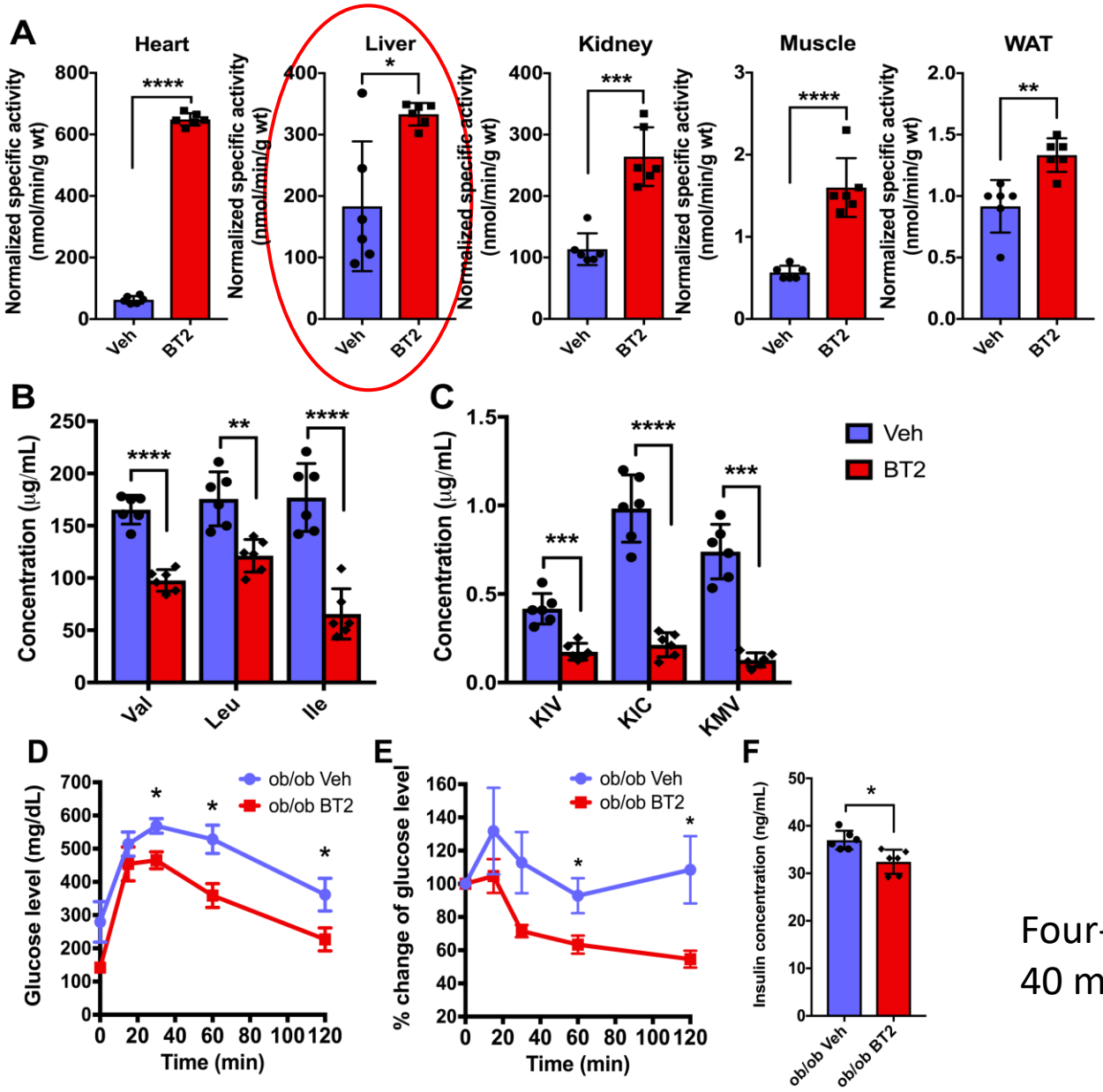
Tso *et al.* 2014, *JBC* **289**, 20583

	S-CPP	BT-2
Plasma pharmacokinetics		
Dose	40 mg/kg IP	10 mg/kg IP
Terminal $t_{1/2}$	127 min	730 min
C_{max}	88,067 ng/ml	71,600ng/ml
T_{max}	10 min	30 min
AUC_{last}	8,354,759 min·ng/ml	69,834,000 min·ng/ml
V_z/F	20.8 ml	3.80 ml
L/F	0.113 ml/min	0.00361 ml/min
Stability in S9 fraction		
$t_{1/2}$	187 min	>> 240 min
Plasma protein binding		
Fraction bound	72.8 %	99.3 %

Morlock and Williams

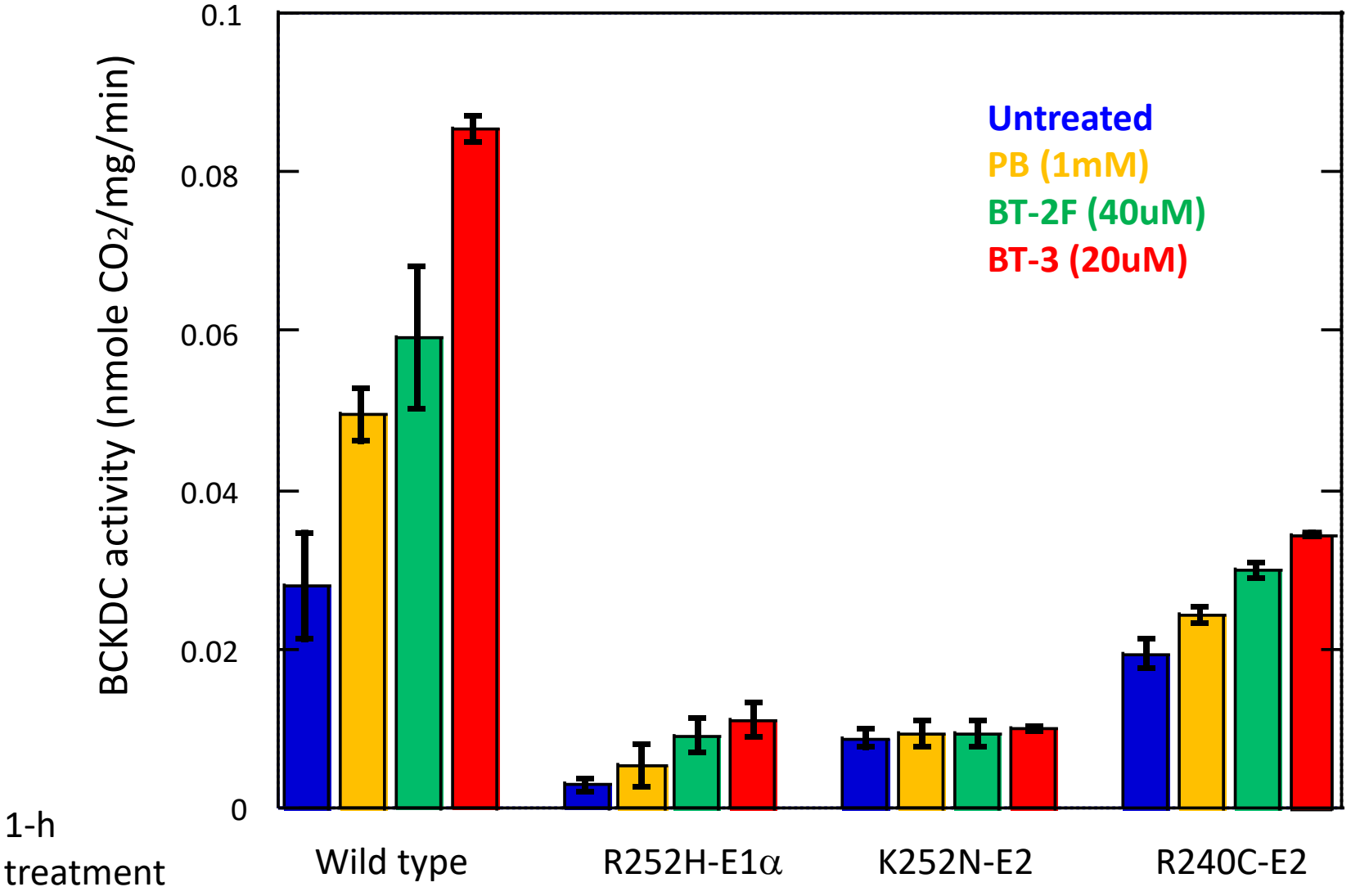

Tyr99
His132
Ile72
OH
HN
N
Leu106
Cl
O
NH2
N
H2
N−R167
+
Cl
S
O
Val125
Leu129
NH2
H2N
N−R171
+
= available space to grow inhibitor

BT2 treatment reduces BCAAs/BCKAs and improves glucose and insulin tolerance in leptin-deficient *ob/ob* mice

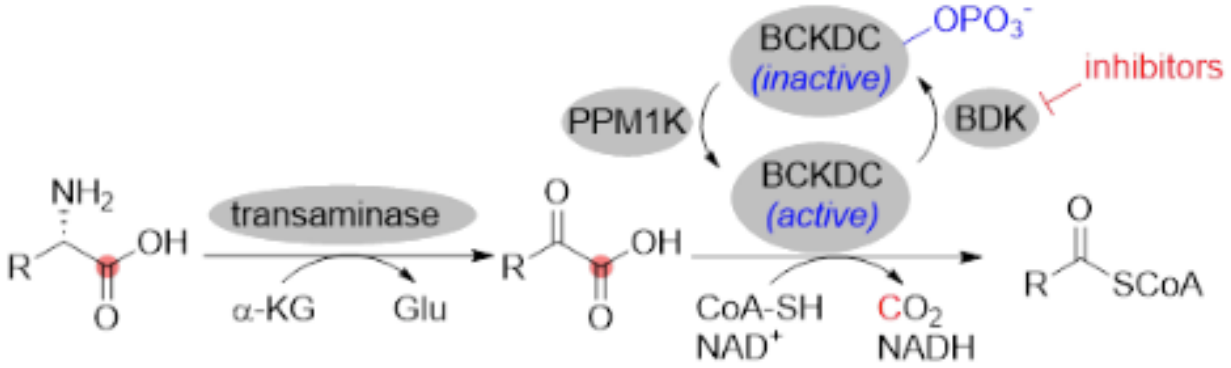


Four-week treatment
40 mg/kg/day

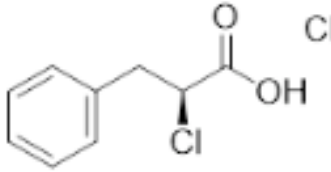
Partial restoration of BCKDC activity in BDK inhibitor-treated lymphoblasts from MSUD patients



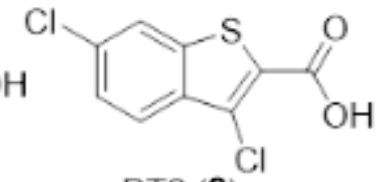
a. Metabolism of branched chain amino acids



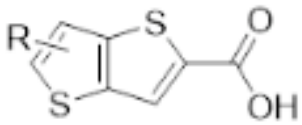
b. Inhibitors of BDK



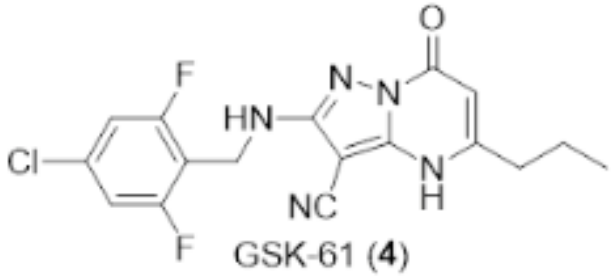
1
IC_{50} = 6.3 μM



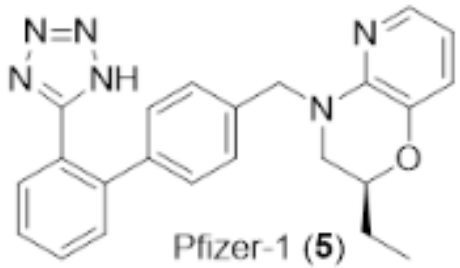
BT2 (**2**)
IC_{50} = 3.2 μM



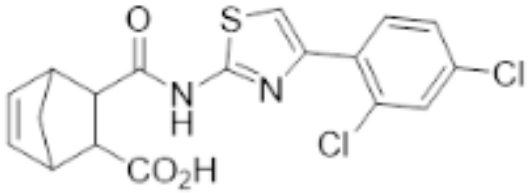
Pfizer-2020 (**3**)
IC_{50} ~ 1 μM



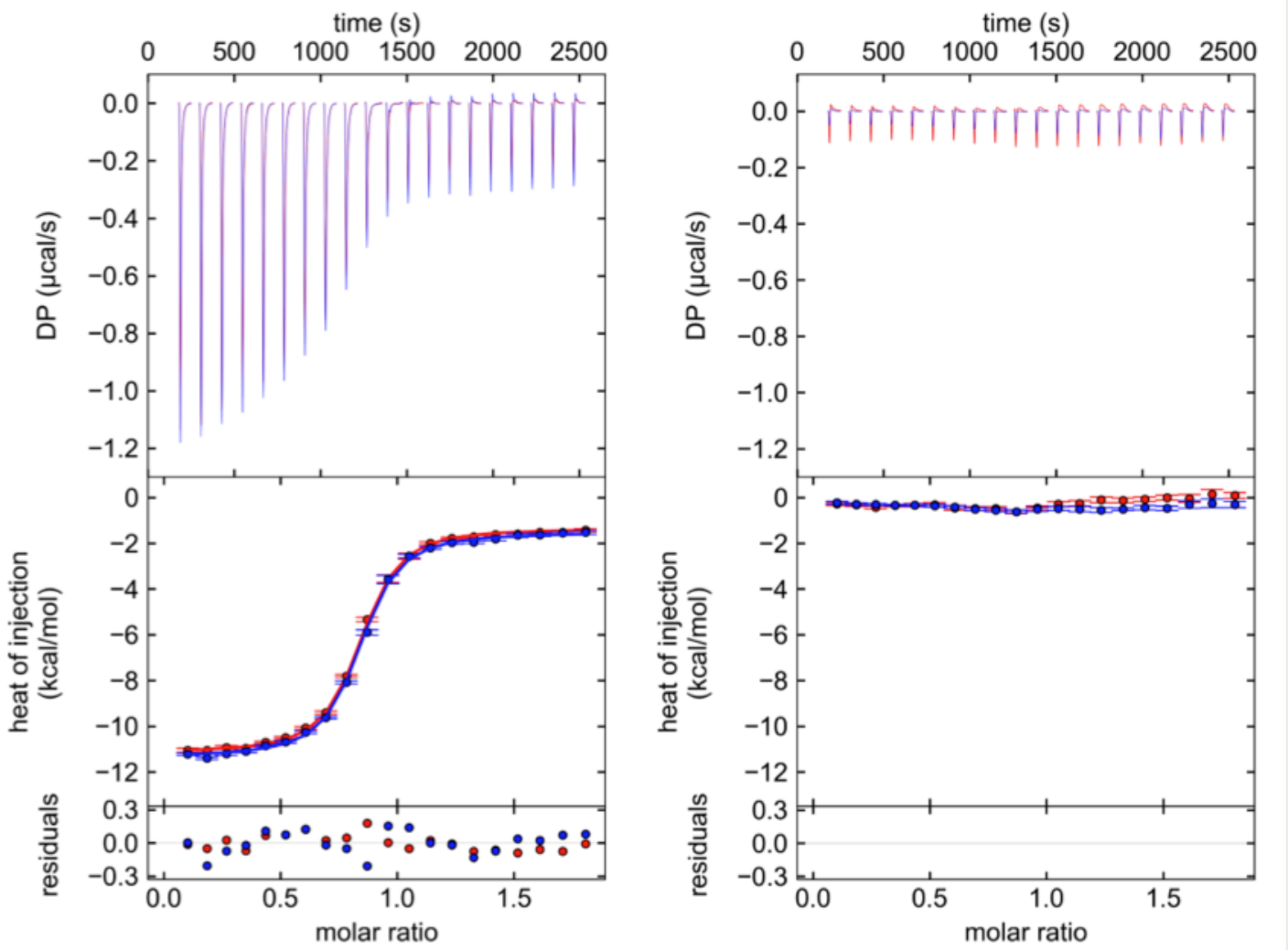
GSK-61 (**4**)
IC_{50} = 8 μM



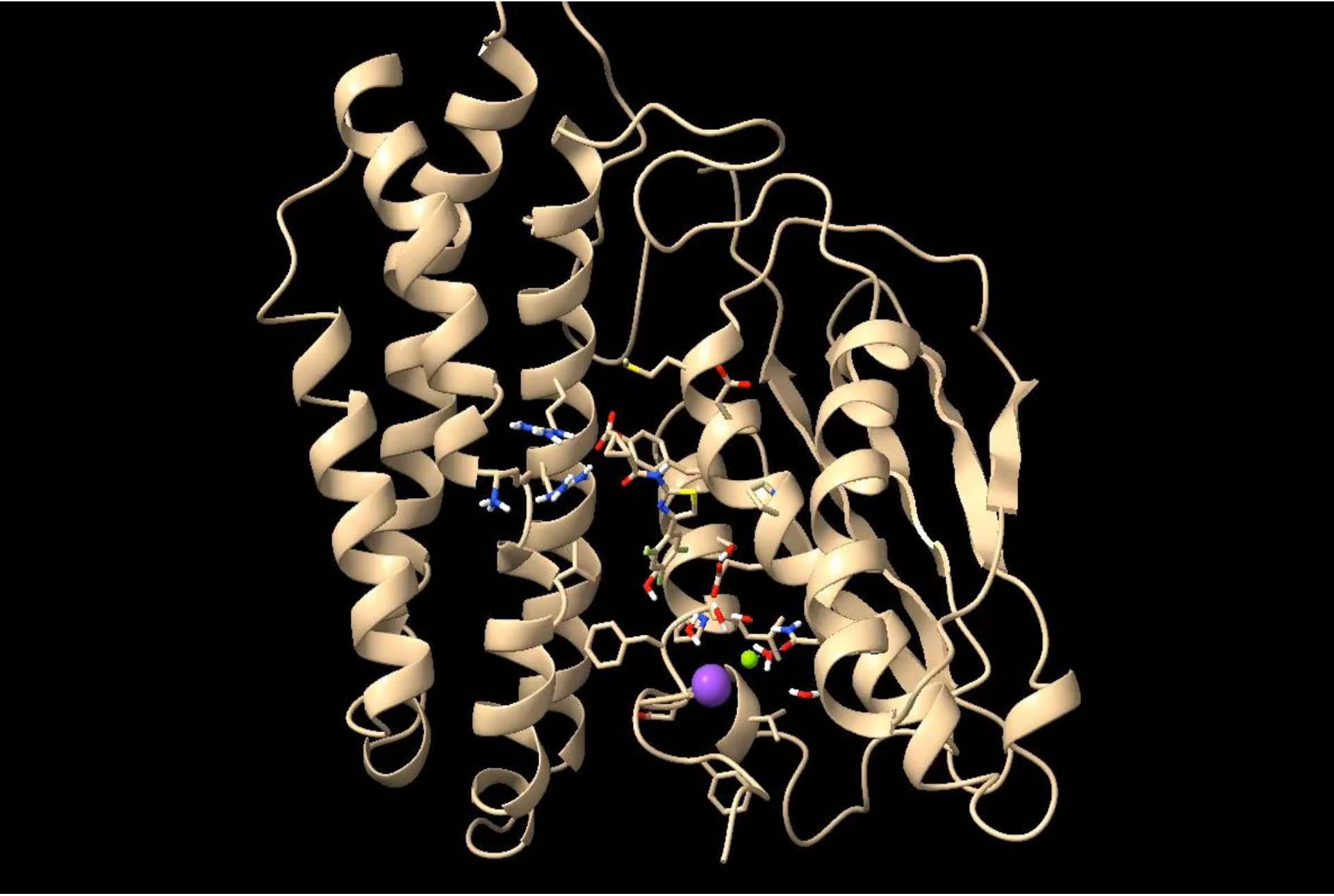
Pfizer-1 (**5**)
IC_{50} = 6 nM



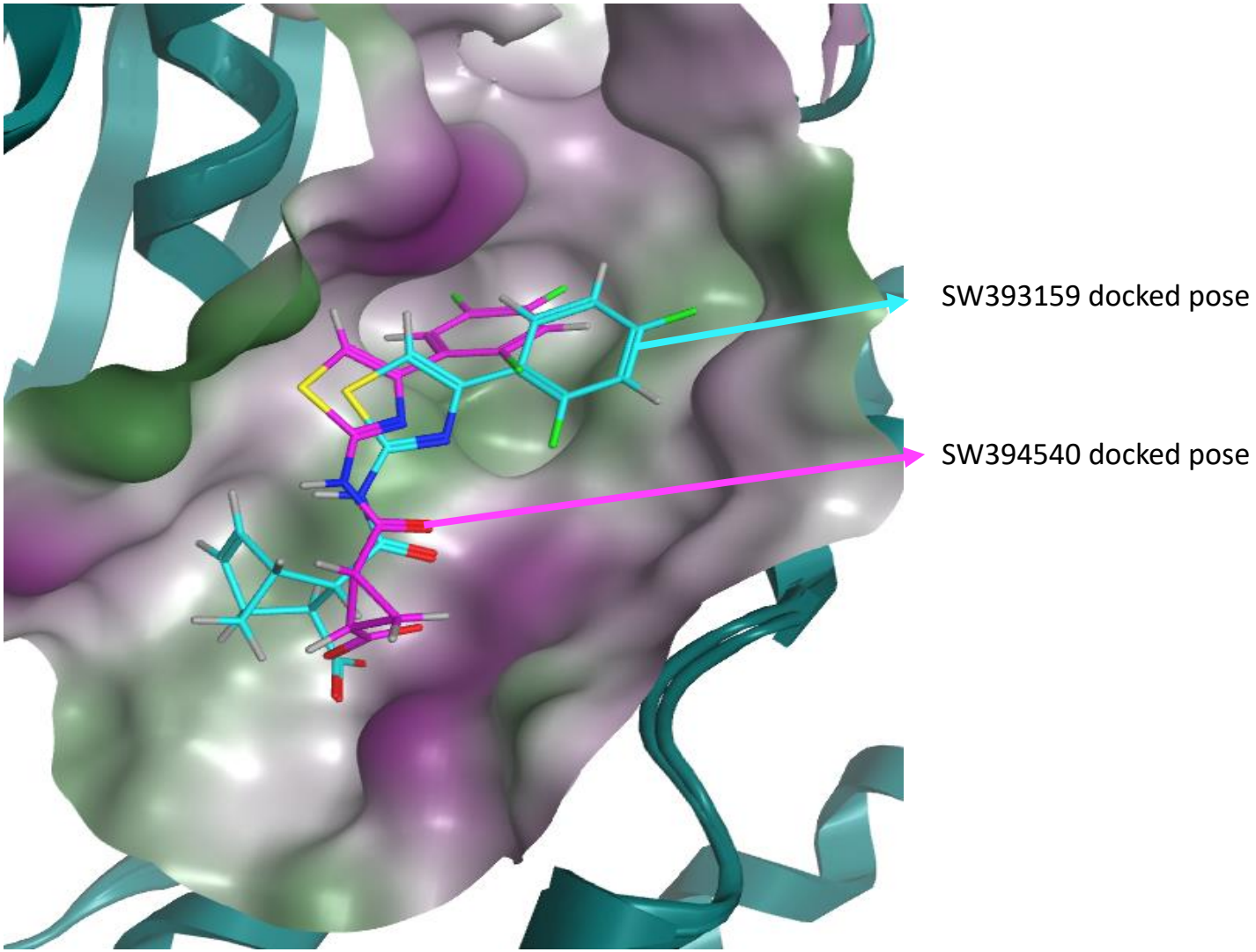
HTS hit (**6**)
IC_{50} = 230 nM



K_d = 480 nM, delta H = -9.81 kcal/mol, delta S = -4.55 cal/mol*K measured for SW394921-1



Overlay of SW393159 and active crystal SW394540 docked pose



Summary

- There is a growing recognition that BCAA catabolism, BDK activity in particular, also plays an important role in several major human diseases including cardiometabolic diseases and MSUD.

- BCAA homeostasis in normal development and human health has been clearly demonstrated, however there are no FDA approved therapies currently on the market that are specifically targeted to BCAA catabolic flux or targeted to the BDK.

- Multiple pharma-companies have increased interest in cardiometabolic diseases and MSUD. The landscape remains rich for development of such therapies.

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the orphan disease center

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Acknowledgments

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Structural Biology Lab

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Chen Gao

Baylor College of Medicine

Brendan Lee

Clinic for Special Children

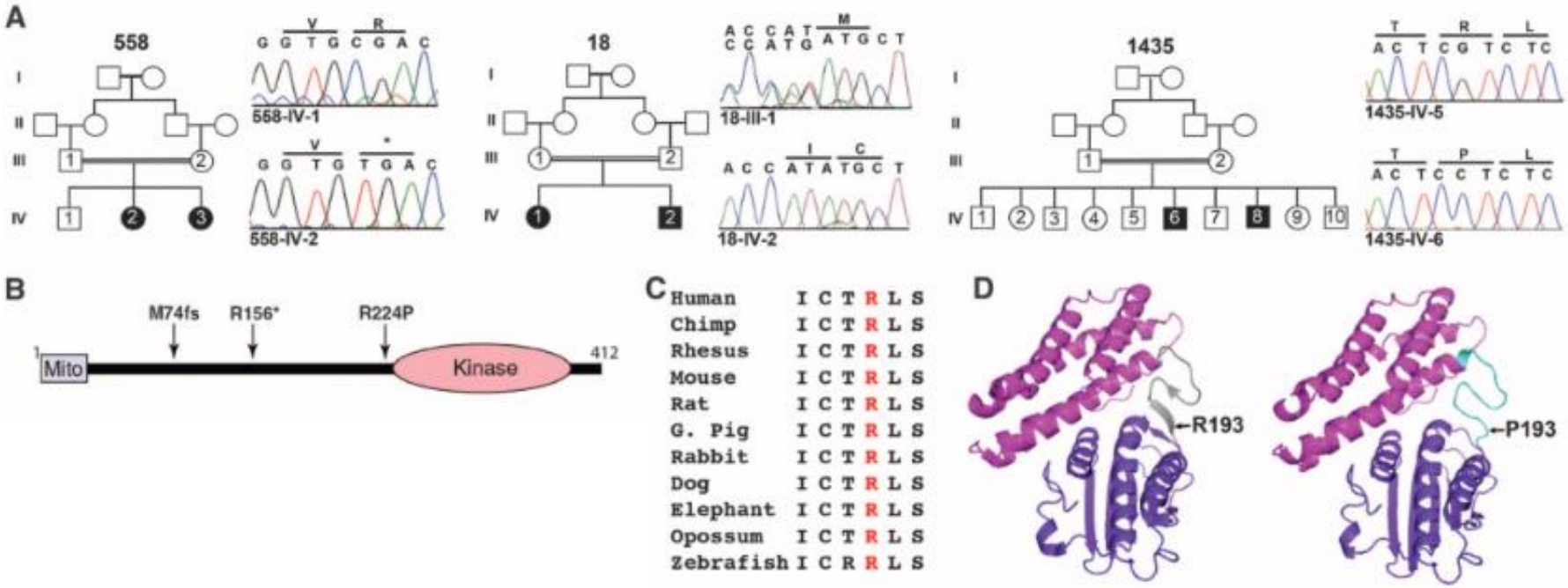
Kevin Strauss

UMass

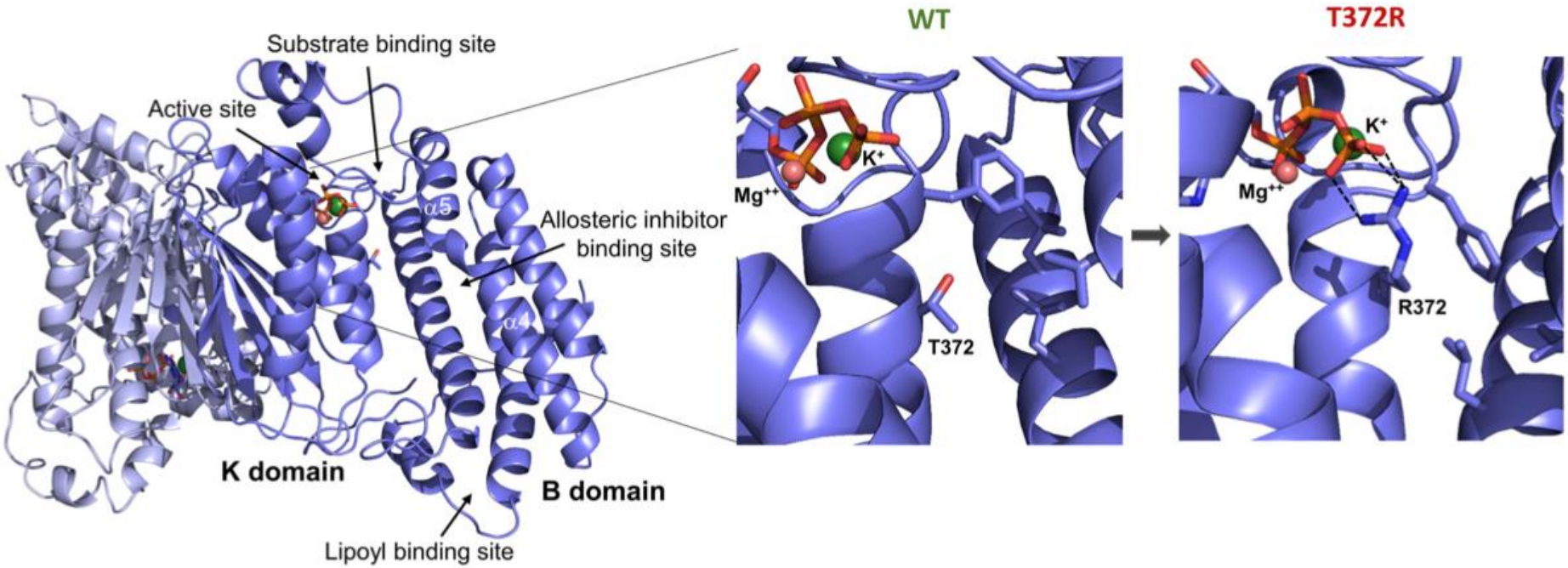
Dan Wang
Jiaming Wang
Heather Gray-Edwards

MSUD Family Support Group

MSUD SAB



Novarino, G., et al., Science 2012



Singh, E., et al., JIMD Reports 2024