Tutorial for

KEGG Online Database

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Acknowledgment:

Thanks to Dr. Peter Abuja for his expert knowledge in the area of methabolisms and pathways.

This document was created by Michael Kalkusch after a meeting on 17th August 2006 with Dr. Peter Abuja and Marc Streit.

This document is available at:

Snapshots from KEGG web interface

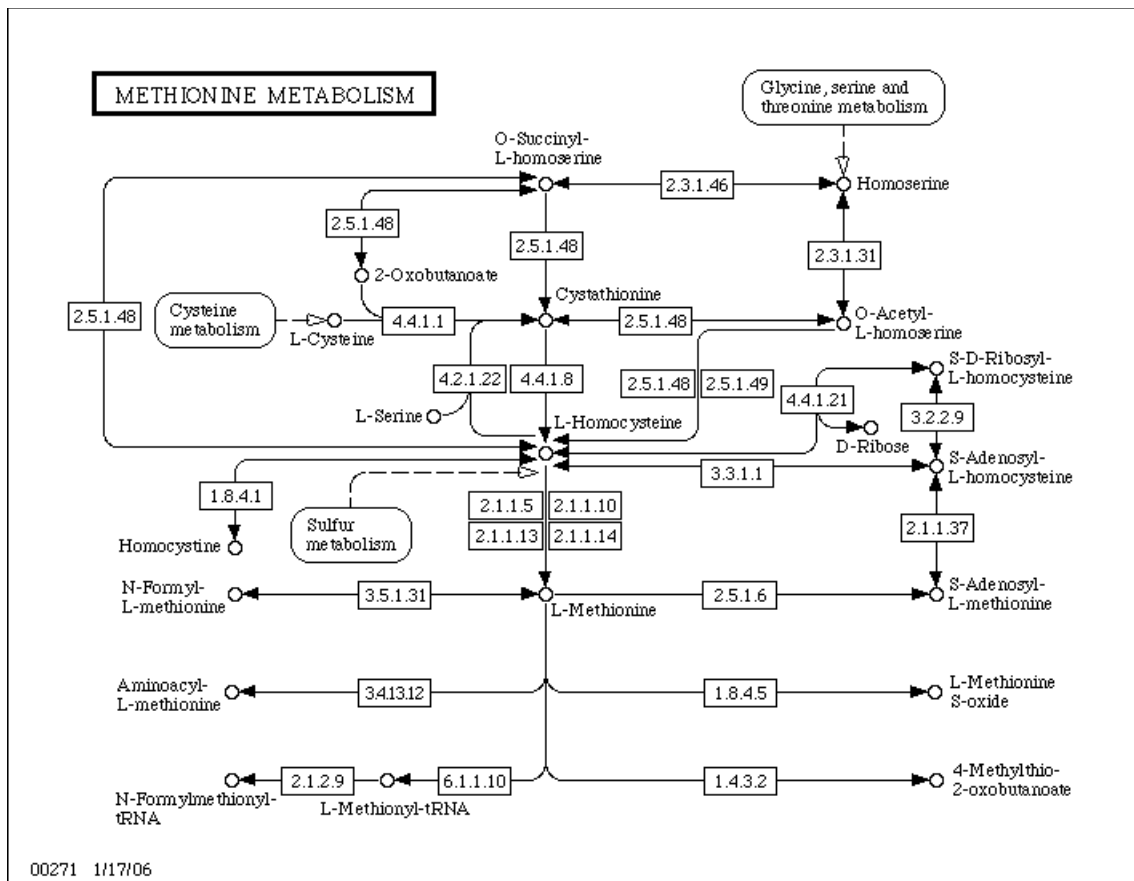Example: Pathway „*Methionine metabolism - Reference pathway* „

Figure 1

Klick on [2.5.1.48] opens this:

KEGG ENZYME: 2.5.1.48

Help

Entry   EC 2.5.1.48   Enzyme

Name   cystathionine gamma-synthase;
                O-succinyl-L-homoserine succinate-lyase (adding cysteine);
                O-succinylhomoserine (thiol)-lyase;
                homoserine O-transsuccinylase;
                O-succinylhomoserine synthase;
                O-succinylhomoserine synthetase;
                cystathionine synthase;
                cystathionine synthetase;
                homoserine transsuccinylase

Class   Transferases
                Transferring alkyl or aryl groups, other than methyl groups
                Transferring alkyl or aryl groups, other than methyl groups

Sysname   O4-succinyl-L-homoserine:L-cysteine
                S-(3-amino-3-carboxypropyl)transferase

Reaction   O-succinyl-L-homoserine + L-cysteine = L-cystathionine + succinate
                [RN:R00999 R01288 R02508 R03132 R03260 R04944 R04945 R04946]

Substrate   O-Succinyl-L-homoserine [CPD:C01118];
                L-Cysteine [CPD:C00097]

Product   L-Cystathionine [CPD:C02291];
                Succinate [CPD:C00042]

Cofactor   Pyridoxal phosphate [CPD:C00018]

Comment   A pyridoxal-phosphate protein. Also reacts with hydrogen sulfide and
                methanethiol as replacing agents, producing homocysteine and
                methionine, respectively. In the absence of thiol, can also catalyse
                beta,gamma-elimination to form 2-oxobutanoate, succinate and
                ammonia.

Pathway   PATH: map00271 Methionine metabolism
                PATH: map00272 Cysteine metabolism
                PATH: map00450 Selenoamino acid metabolism
                PATH: map00920 Sulfur metabolism

Ortholog   KO: K01739 cystathionine gamma-synthase

Genes   XLA: 494673(LOC494673)
                XTR: 394634(MGC75946)
                ATH: At3g01120(T4P13.19)
                CME: CMF156C
                SCE: YJR130C(STR2) YML082W
                AGO: AER164C(AER164Cp)
                CAL: orf19.1033(STR2) orf19.7297
                SPO: SPBC15D4.09c
                CNE: CNC01220
                LMA: LmjF35.3230
                EHI: 132.t00018 389.t00003 395.t00003
                ECO: b3939(metB)
                ECJ: JW3910(metB)
                ...: reduced the list of 191 gene in total

Reference   1 [PMID:5340123]
                Flavin M, Slaughter C.
                Enzymatic synthesis of homocysteine or methionine directly from
                O-succinyl-homoserine.
                Biochim. Biophys. Acta. 132 (1967) 400-5.

```
2  [PMID:5922970]
Kaplan MM, Flavin M.
Cystathionine gamma-synthetase of Salmonella. Catalytic properties
of a new enzyme in bacterial methionine biosynthesis.
J. Biol. Chem. 241 (1966) 4463-71.
3  [PMID:6016326]
Wiebers JL, Garner HR.
Homocysteine and cysteine synthetases of Neurospora crassa.
Purification, properties, and feedback control of activity.
J. Biol. Chem. 242 (1967) 12-23.
4
Wiebers, J.L. and Garner, H.R. Acyl derivatives of homoserine as
substrates for homocysteine synthesis in Neurospora crassa, yeast,
and Escherichia coli. J. Biol. Chem. 242 (1967) 5644-5649.
5  [PMID:9843488]
Clausen T, Huber R, Prade L, Wahl MC, Messerschmidt A.
Crystal structure of Escherichia coli cystathionine gamma-synthase
at 1.5 A resolution.
EMBO. J. 17 (1998) 6827-38.
6  [PMID:9531508]
Ravanel S, Gakiere B, Job D, Douce R.
Cystathionine gamma-synthase from Arabidopsis thaliana: purification
and biochemical characterization of the recombinant enzyme
overexpressed in Escherichia coli.
Biochem. J. 331 ( Pt 2) (1998) 639-48.
```

Other DBs   IUBMB Enzyme Nomenclature: 2.5.1.48
            ExPASy - ENZYME nomenclature database: 2.5.1.48
            ERGO genome analysis and discovery system: 2.5.1.48
            BRENDA, the Enzyme Database: 2.5.1.48
            CAS: 9030-70-0

LinkDB   [All DBs]

=> **Original format**

DBGET integrated database retrieval system, GenomeNet

Figure 2

Klick on pathway „PATH: map00271   Methionine metabolism" shows this:

Figure 3

Klick on Cystathionine opens this page:

KEGG COMPOUND: C02291

Help

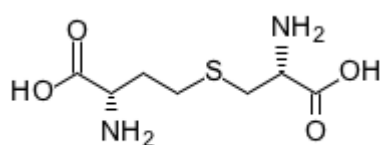|		
Entry	C02291	Compound

Name L-Cystathionine

Formula C7H14N2O4S

Mass 222.0674

Structure

C02291

Mol file   KCF file   DB search

Reaction R01001 R01286 R01290 R03217 R03260

Pathway PATH: map00260 Glycine, serine and threonine metabolism
PATH: map00271 Methionine metabolism

Enzyme 2.5.1.48   2.5.1.49   4.2.1.22   4.4.1.1
4.4.1.8

Other DBs CAS: 56-88-2
PubChem: 5347
ChEBI: 17482

LinkDB All DBs

KCF data Show

=> **Original format**

DBGET integrated database retrieval system, GenomeNet
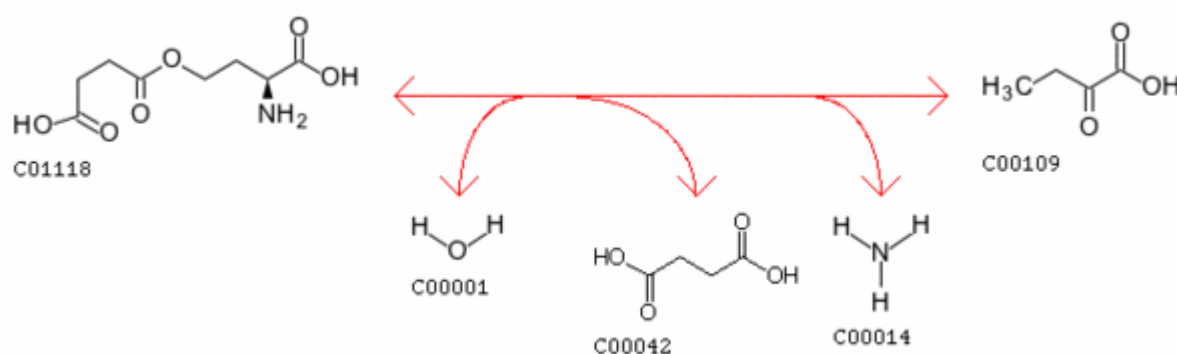
Figure 4

Example for a reaction:

REACTION: R00999

Help

Entry	R00999	Reaction

Name   O-Succinyl-L-homoserine succinate-lyase (adding cysteine)

Definition O-Succinyl-L-homoserine + H2O <=> 2-Oxobutanoate + Succinate + NH3

Equation   C01118 + C00001 <=> C00109 + C00042 + C00014

RPair   RP: A00132   C00042_C01118 main
          RP: A01229   C00109_C01118 main

Pathway   PATH: rn00271   Methionine metabolism

Enzyme   2.5.1.48

Ortholog KO: K01739   cystathionine gamma-synthase

LinkDB   All DBs

=> **Original format**

DBGET integrated database retrieval system, GenomeNet

Figure 5
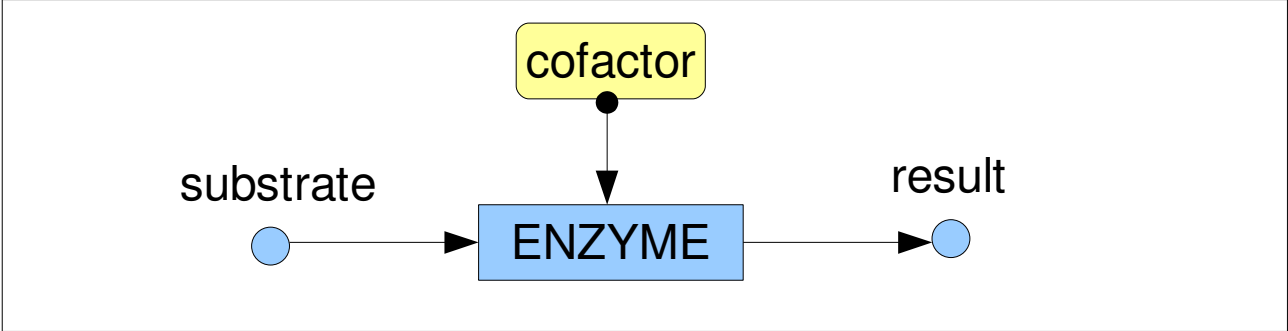
Flow Charts:

Enzyme:

Figure 6

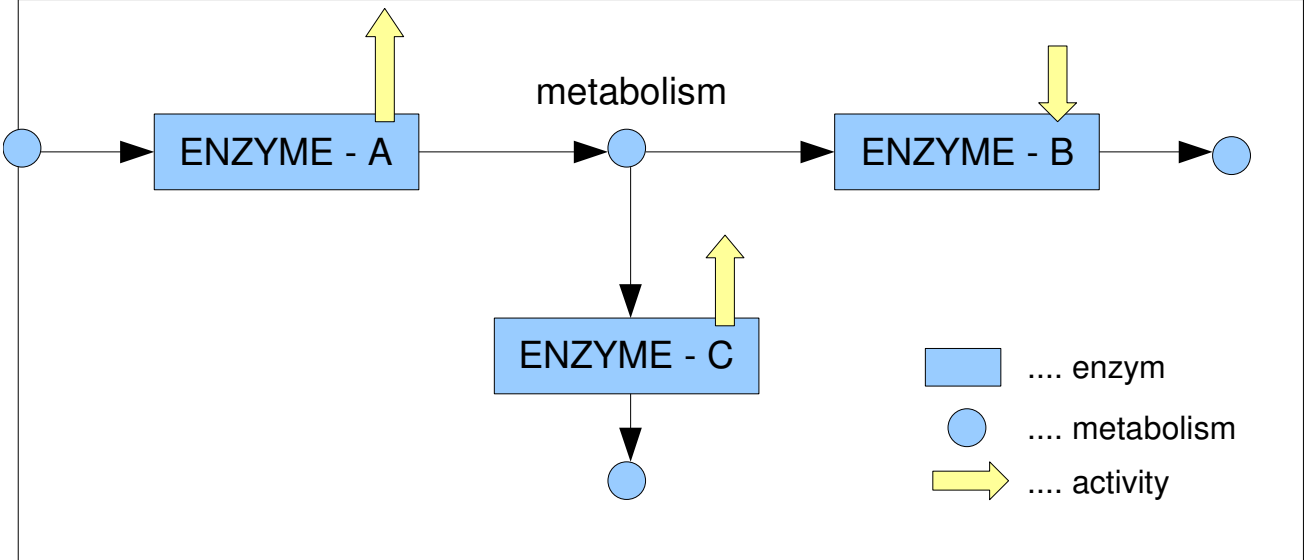Regulation of enzymes and metabolism

Figure 7

Concepts:

- Inside pathway: 2 step, 3 step relations inside the graph fram a given Protein or Methabolit

- Take connectivity of methabolit into account (i.e. google principle)

- Use connectivity of methabolit as wight for its importance inside pathways
  (Spring mass modell / Graviation modell)

- Interrelations/ interaction:
    - Kinase ==> Protein
    - Protein <==> Protein interrelations

- Visualize flows from:

    Substrate ==>
    (Enzyme/ Protein) <-- using cofactor   ==>
    Methabolit A ==>
    (Enyzm / Protein) ==>
    Methabolit B

A possibility here could be the application of Petri-Nets (http://en.wikipedia.org/wiki/Petri_net)

- Semantic Zooming:
    - hide / show methabolits
    - collapse protein chains
    - collapse pathway inside another pathway
    - magic lense for sematic zooming

- Messure activity of Methabolids:
  required for detailed analysis, but not done within Micro Array analysis
- Position of enzyme inside cell
- Compartments the enzyme is present in
- Handle enzyme casscades ==> if top element of enzyme-casscade is active, the casscade might
  be active to. Thus only the result of the casscade is active, but not its intermediate enzyms.

- Important fields of „Enzymes" in KEEG (see figure 2):

  Entry: unique international standardized identifyier
  Name: known names for this molecule and aliases for it
  Reaction: chemical reaction
  Substrate: Input to the Enzyme
  Product: Output of the Enzyme
  Cofactor: if set, it is nesseccary to activate the Enzyme
  Pathway: shows only active pathways linked to one Enzyme but not pathways influenced by this enzyme!
  Ortholog: special genes (see glossary)
  Genes: ???

- Important fields of „Compounds" in KEGG (see figure 4):

  Entry: unique code (used by KEGG? Only or also international unique?)
  Name: known names for this molecule and aliases for it
  Mass: mass of this molecule (mass unit?)
  Structur: chemical structure of thie molecule
  Reaction: chemical reaction, were this compound is part of
  Pathway: list of active pathways, were this compound can be found
  Enzyme: list of enzymes capeable of handling this compound, either as input or as output; also know as „Substrate" and „Product"

- Important fields of „Reaction" in KEGG (see figure 5):

  Entry: unique name (probably only in KEGG)
  Name: name that defines this reaction
  Definition: define this reaction
  Equation: link to compunds of this reaction
  Rpair: ??
  Pathway: list of pathways were this reaction is part of
  Enzyme: list of enzymes that do this reaction
  Ortholog: special genes (see glossary)

Other databases aside from KEGG:

(suggested by Dr. Abuja)

Biomodal DB: http://www.ebi.ac.uk/biomodals/

Reactome „A curated knowledgebase of biological pathways"   http://www.reactome.org/

Brookhaven Protein Database   http://cds.dl.ac.uk/cds/pdb.html
    for structure of enzymes

EMBL „European Molecular Biology Laboratoy"   http://www.embl.org/

EMBO „European Molecular Biology Organization"   http://www.embo.org/

Other important websites:

RCSB „Research Collaboratory for Structural Bioinformatics "   http://home.rcsb.org/

RCSB PDB Protein Data Base   http://www.rcsb.org/pdb/

Software we should look at:

- „Panther" (Applied Biosoftware)
- google: „Pathway" as well as „Pathway Heatmap"

Books:

David Fell, „Regulation of Metabolism" („Kinetische Regulation")

Homepage of David Fell:

http://www.brookes.ac.uk/bms/research/fell.html

Glossary:

Term	Description	Link
Substrate	a molecule upon which an enzyme acts. Enzymes catalyze chemical reactions involving the substrate(s).	http://en.wikipedia.org/wiki/Substrate_(biochemistry)
Kinase	a type of enzyme that transfers phosphate groups from high-energy donor molecules, such as ATP, to specific target molecules (substrates); the process is termed *phosphorylation*.	http://en.wikipedia.org/wiki/Kinase
Inhibitor	???	
Effektor	???	
Kinetische Regulation	???	
EC-Number	Enzyme Commission number (EC number)	http://en.wikipedia.org/wiki/EC_number
Homocysteine		http://en.wikipedia.org/wiki/Homocysteine
Ortholog	**Orthologs** are genes in different species which evolved from a common ancestral gene.	http://en.wikipedia.org/wiki/Ortholog