# Solute Transport(ers) in Zebrafish Gut

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ZF-MED - Zebrafish and other aquatic models in Mediterranean Labs

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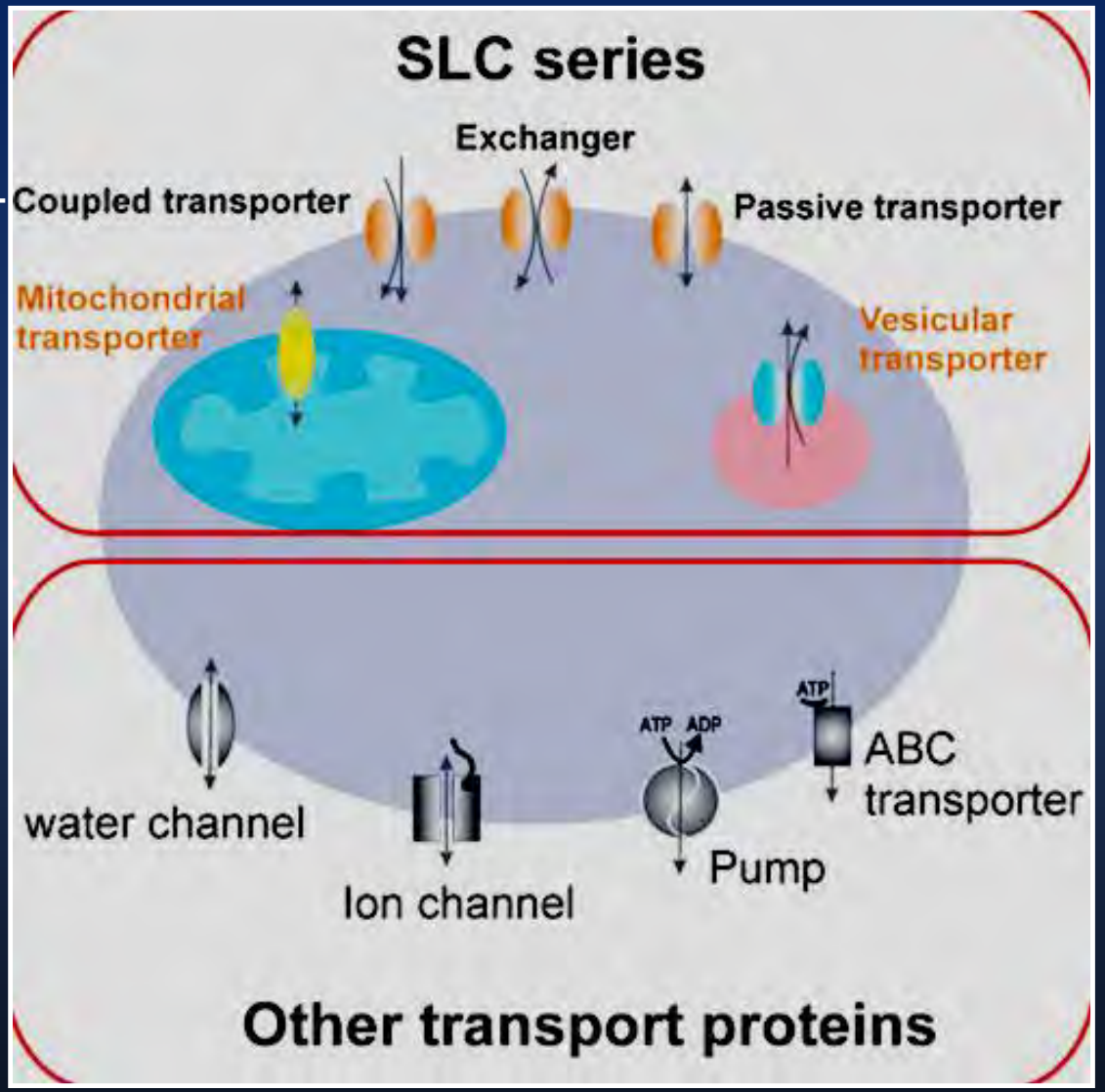
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' - Teramo

# The Solute Carrier (SLC) family series

# The HUGO Solute Carrier (SLC) family series




***The SLC series includes passive transporters, ion-coupled transporters and exchangers***

***Passive transport***

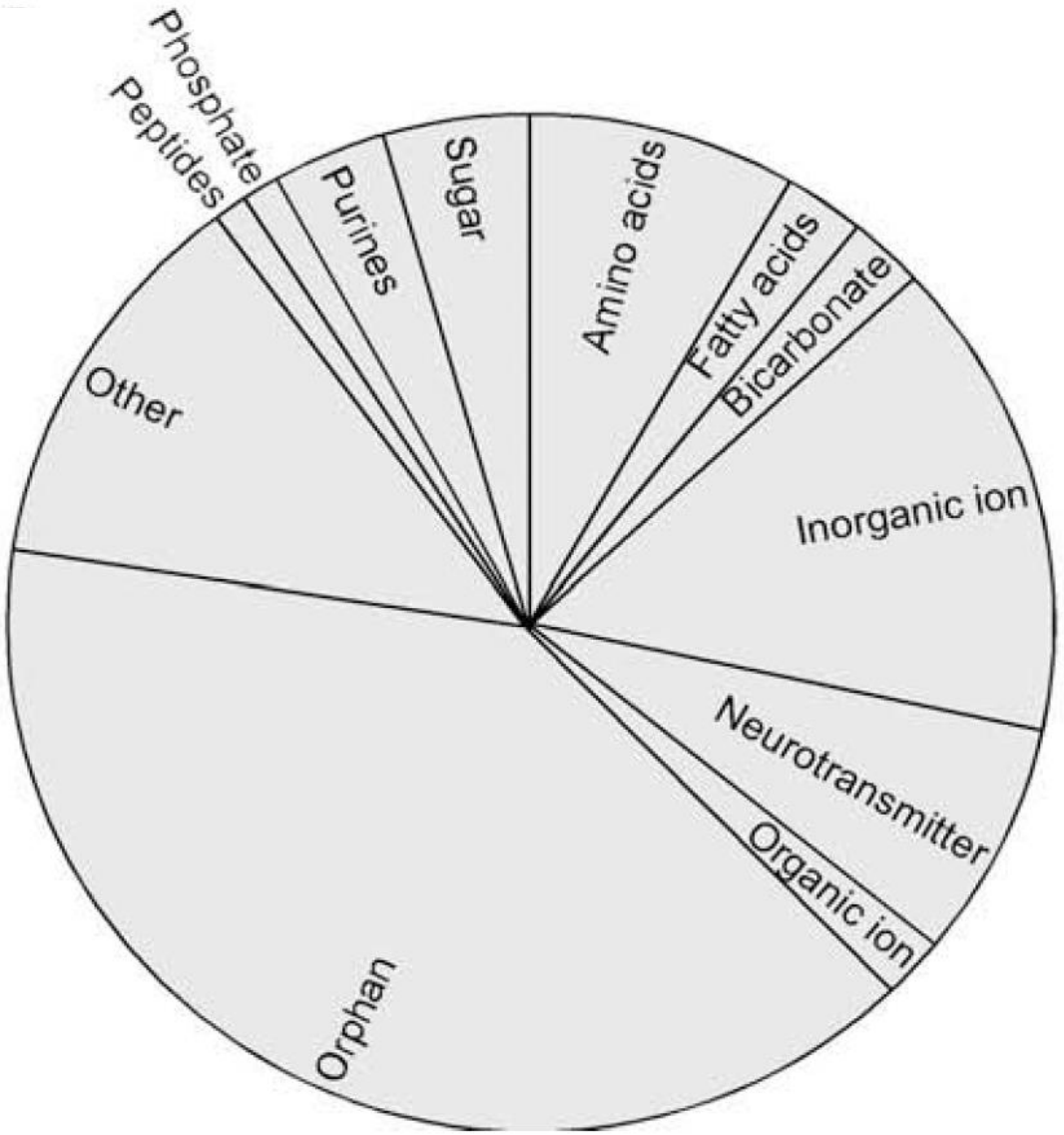
***A kind of transport by which ions or molecules move along a concentration gradient, which means movement from an area of higher concentration to an area of lower concentration.***

***Coupled transport***

***The linked, simultaneous transport of two substances across a cell membrane (or another intracellular membrane). If the two substances are moving in the same direction (both into the cell or both out of the cell) it is called symport. If the two substances are moving in opposite directions (one moves into the cell while the other moves out) it is called antiport.***

Adapted from:
Hediger et al., Pflügers Arch. 447:485 (2004)

# The HUGO Solute Carrier (SLC) family series

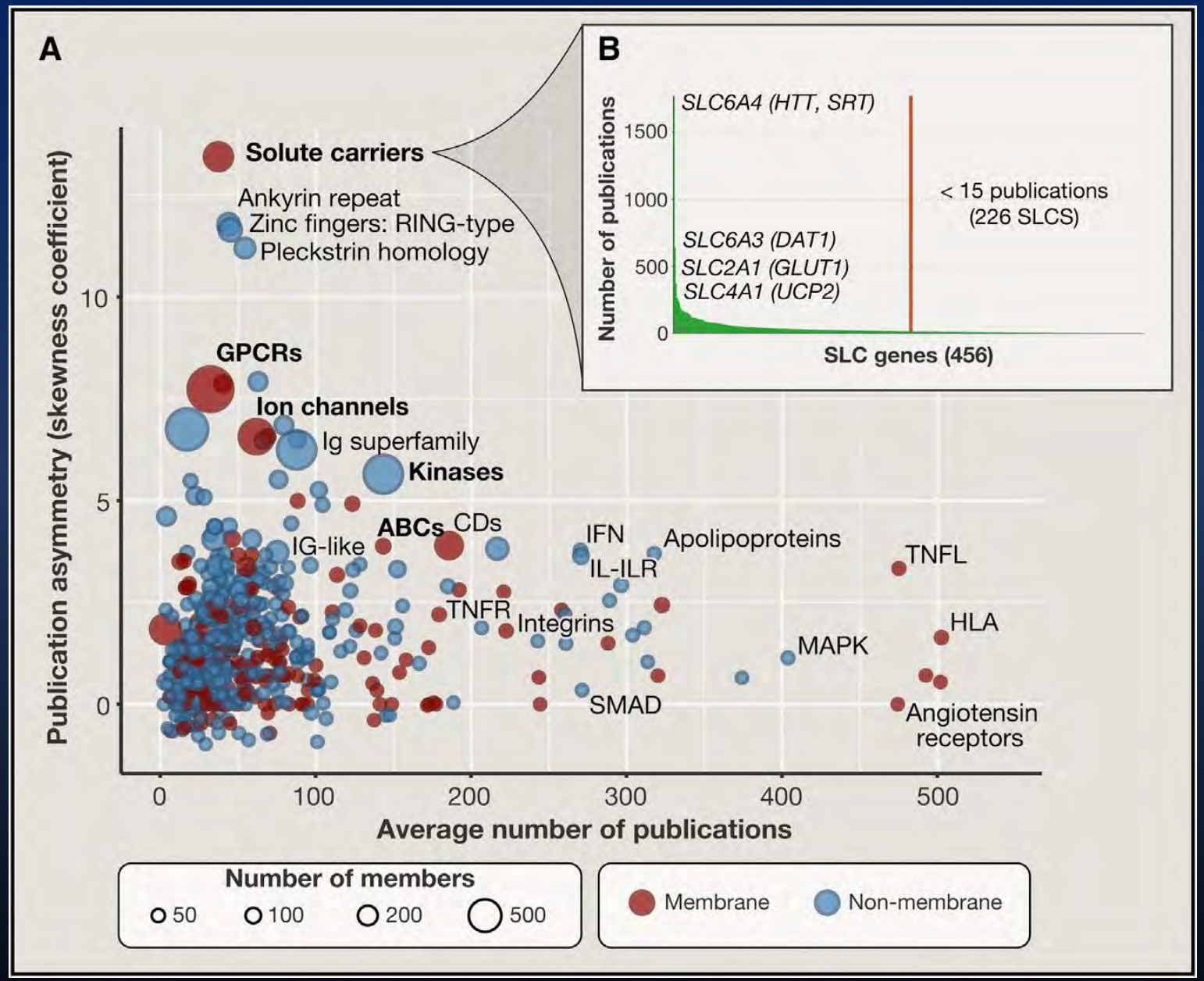


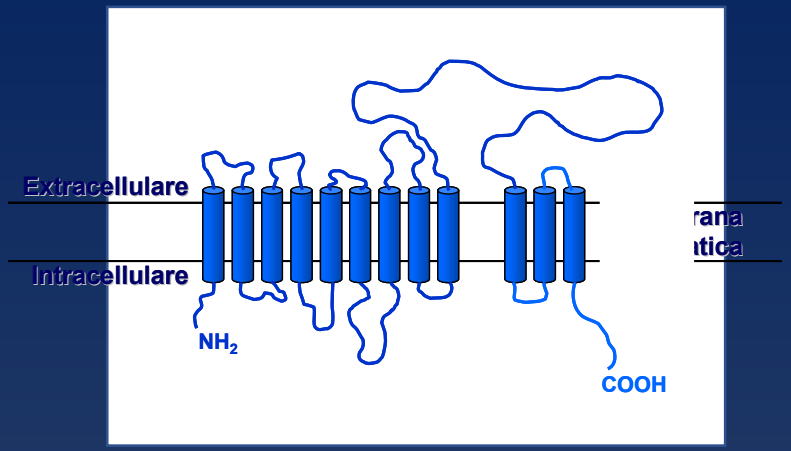

***SLCs classification based on the type of substrate they are transporting.***

*The transporters were classified into ten major groups based on the substrate they are transporting according to the literature. Orphan transporter (substrate unknown) and other (substrate does not fit into any of the ten major categories) are also included as groups in the graph.*

Adapted from:
Fredriksson et al., FEBS Lett. 582:3811 (2008)

# The HUGO Solute Carrier (SLC) family series




***After G-protein-coupled receptors (GPCRs), SLCs are the second-largest family of membrane proteins in the human genome. Also, SLCs are the most neglected group of genes in the human genome***

Adapted from:
Cesar-Razquin et al., Cell 162:478 (2015)

# The HUGO Solute Carrier (SLC) family series

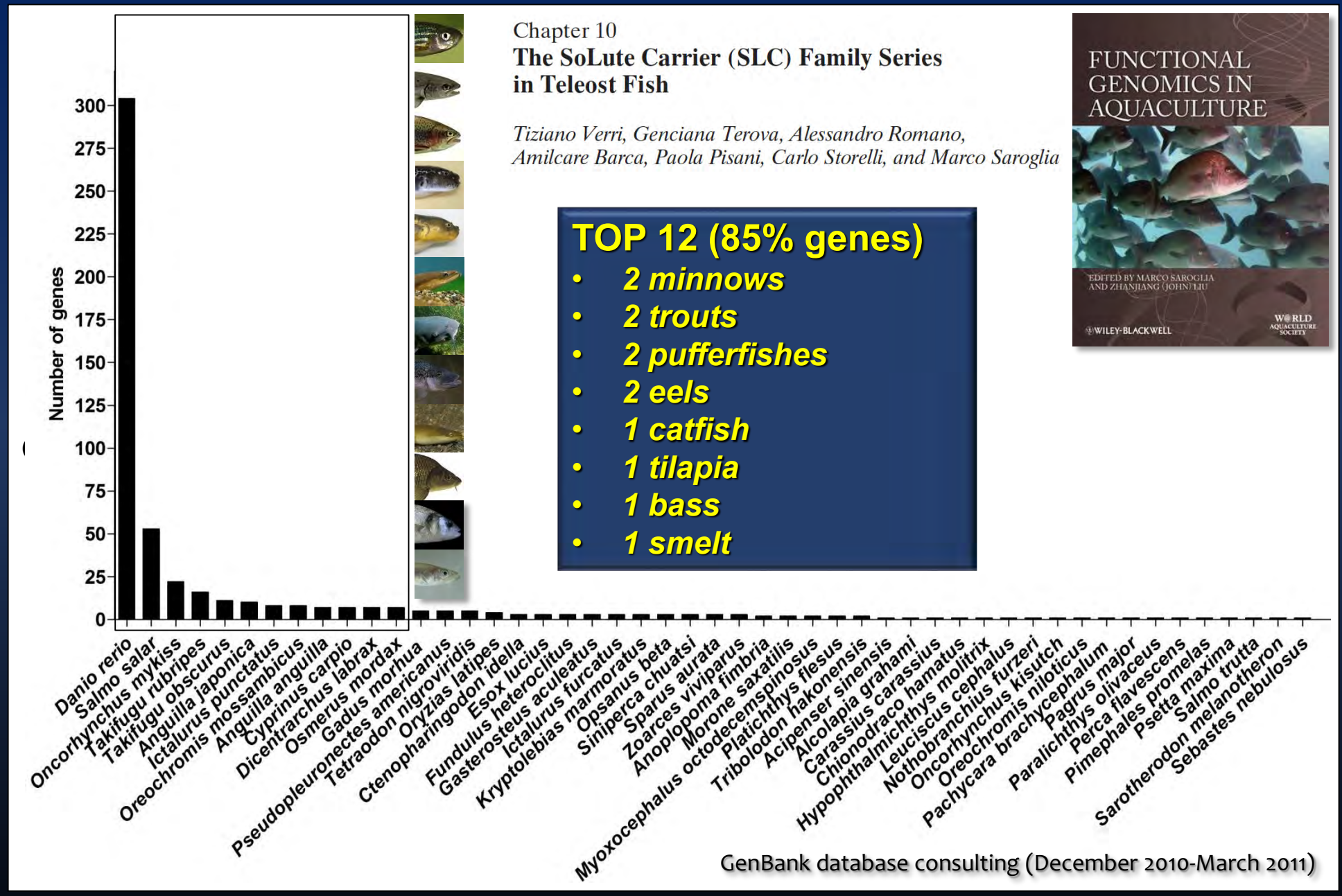

**65 families**
**458 genes**

[+] SLC1 High-affinity glutamate and neutral amino acid transporter family
[+] SLC2 Facilitative GLUT transporter family
[+] SLC3 Heavy subunits of the heteromeric amin
[+] SLC4 Bicarbonate transporter family
[+] SLC5 Sodium glucose cotransporter family
[+] SLC6 Sodium- and chloride-dependent neurot
[+] SLC7 Cationic amino acid transporter/glycopr
[+] SLC8 $Na^+/Ca^{2+}$ exchanger family
[+] SLC9 $Na^+/H^+$ exchanger family
[+] SLC10 Sodium bile salt cotransport family
[+] SLC11 Proton-coupled metal ion transporter f
[+] SLC12 Electroneutral cation-coupled Cl cotra
[+] SLC13 Human $Na^+$-sulfate/carboxylate cotrans
[+] SLC14 Urea transporter family
[+] SLC15 Proton oligopeptide cotransporter fam
[+] SLC16 Monocarboxylate transporter family
[+] SLC17 Vesicular glutamate transporter family
[+] SLC18 Vesicular amine transporter family
[+] SLC19 Folate/thiamine transporter family
[+] SLC20 Type III $Na^+$-phosphate cotransporter family
[+] SLC21 Organic anion transporter family
[+] SLC22 Organic cation/anion/zwitterion transporter family
[+] SLC23 $Na^+$-dependent ascorbic acid transporter family
[+] SLC24 $Na^+/(Ca^{2+}\text{-}K^+)$ exchanger family
[+] SLC25 Mitochondrial carrier family
[+] SLC26 Multifunctional anion exchanger family
[+] SLC27 Fatty acid transporter family
[+] SLC28 $Na^+$-coupled nucleoside transport family
[+] SLC29 Facilitative nucleoside transporter family
[+] SLC30 Zinc efflux family
[+] SLC31 Copper transporter family
[+] SLC32 Vesicular inhibitory amino acid transporter family
[+] SLC33 Acetyl-CoA transporter family
[+] SLC34 Type II $Na^+$-phosphate cotransporter family
[+] SLC35 Nucleoside-sugar transporter family
ed amino acid transporter family
hate/phosphate exchanger family
d System N sodium-coupled neutral amino acid
sporter family
on transporter family
gnesium transporter family
m transporter family
lent, system-L-like amino acid transporter family
ransporter family
ansporter family
orter family
d Toxin Extrusion (MATE) family
orter family
d transporter family
[+] SLC50 Sugar efflux transporters
[+] SLC51 Transporters of steroid-derived molecules
[+] SLC52 Riboflavin transporter family
[+] SLC53 Phosphate carriers
[+] SLC54 Mitochondrial pyruvate carriers
[+] SLC55 Mitochondrial cation/proton exchangers
[+] SLC56 Sideroflexins
[+] SLC57 NiPA-like magnesium transporter family
[+] SLC58 MagT-like magnesium transporter family
[+] SLC59 Sodium-dependent lysophosphatidylcholine symporter family
[+] SLC60 Glucose transporters
[+] SLC61 Molybdate transporter family
[+] SLC62 Pyrophosphate transporters
[+] SLC63 Sphingosine-phosphate transporters
[+] SLC64 Golgi $Ca^{2+}/H^+$ exchangers
[+] SLC65 NPC-type cholesterol transporters

Adapted from:
www.bioparadigms.org

# The Solute Carrier (SLC) family series in teleost fish

# The SLC family series in teleost fish




Chapter 10

**The SoLute Carrier (SLC) Family Series in Teleost Fish**

*Tiziano Verri, Genciana Terova, Alessandro Romano, Amilcare Barca, Paola Pisani, Carlo Storelli, and Marco Saroglia*

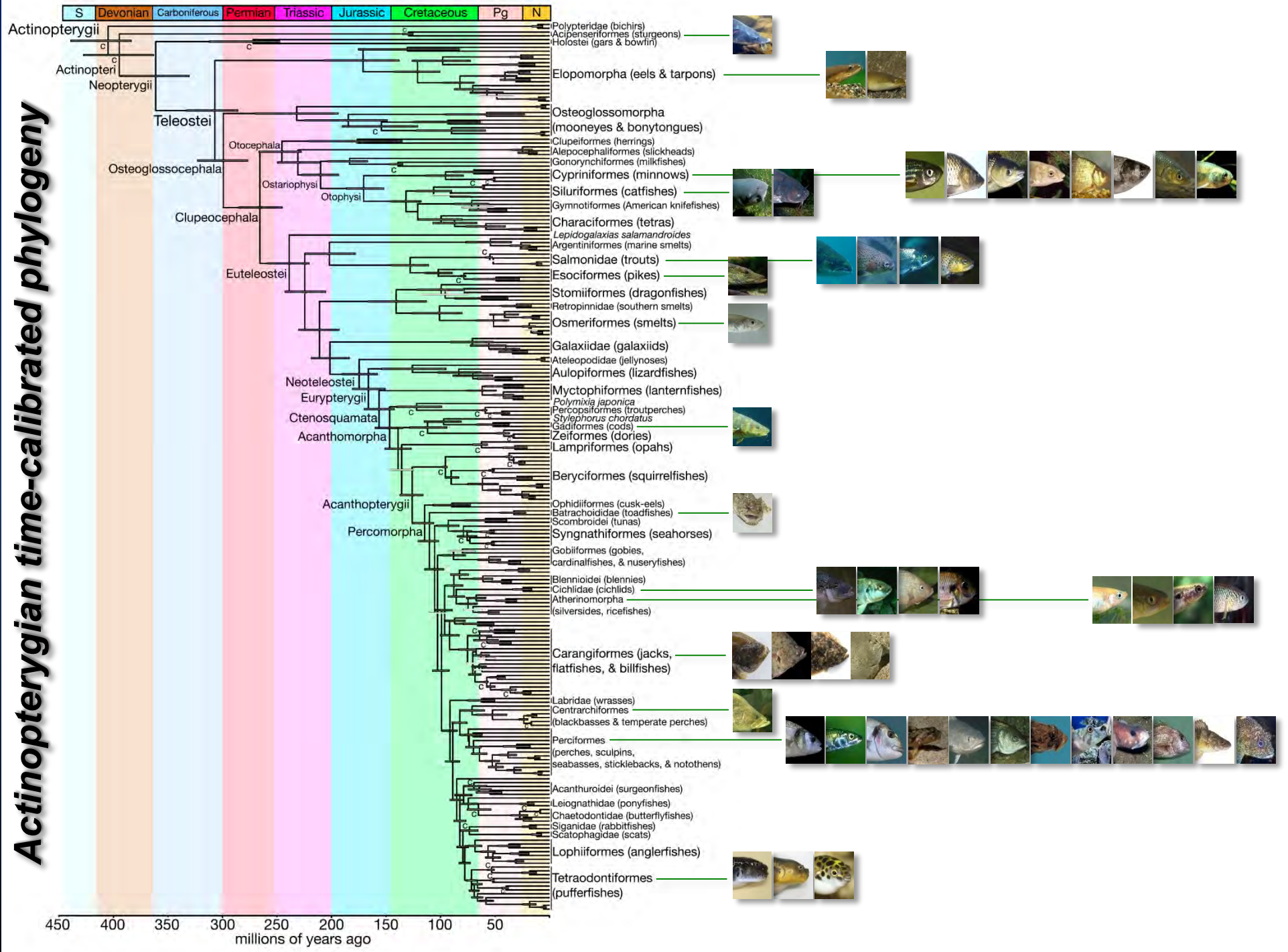



**TOP 12 (85% genes)**

- *2 minnows*
- *2 trouts*
- *2 pufferfishes*
- *2 eels*
- *1 catfish*
- *1 tilapia*
- *1 bass*
- *1 smelt*

GenBank database consulting (December 2010-March 2011)

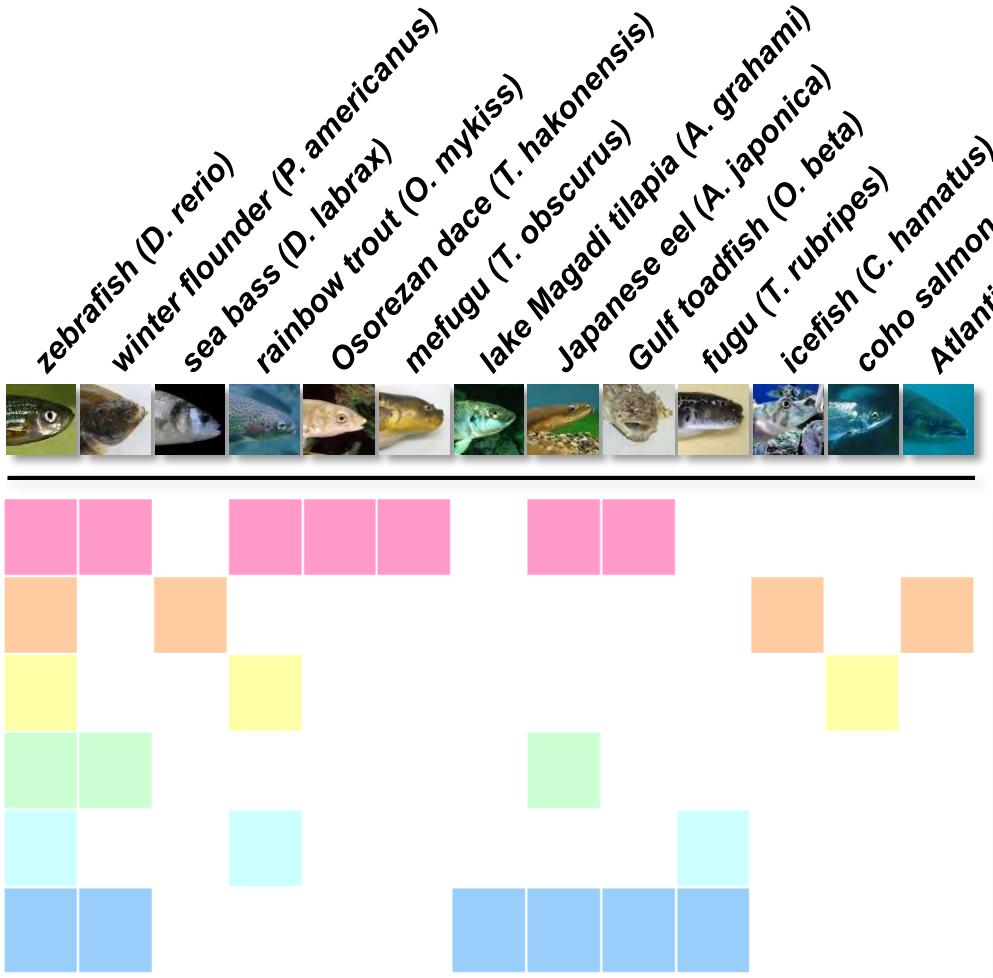
# Actinopterygian time-calibrated phylogeny




Adapted from:
Near et al., *PNAS* 109:13698 (2012)

# The SLC family series in teleost fish

## *Functional expression of SLC proteins in teleost fish*




GenBank database consulting (December 2010-March 2011)

Table 1. List of the solute carrier (SLC) families in zebrafish (*Danio rerio*) compared to human

| Family | Name | Human Total genes | Zebrafish Total genes | Zebrafish Non-duplicated genes | Zebrafish Duplicated genes designated as 'a' and 'b' | Zebrafish Duplicated genes designated as 'tandem duplicate' | Zebrafish Possibly duplicated genes and/or genes not obviously designated |
|---|---|---|---|---|---|---|---|
| SLC1 | High affinity glutamate and neutral amino acid transporter family | 7 | 9 | 5 | 4 | 0 | 0 |
| SLC2 | Facilitative GLUT transporter family | 14 | 12 | 6 | 2 | 0 | 4 |
| SLC3 | Heavy subunits of the heteromeric amino acid transporters | 2 | 2 | 1 | 1 | 0 | 0 |
| SLC4 | Bicarbonate transporter family | 10 | 6 | 3 | 3 | 0 | 0 |
| SLC5 | Sodium glucose cotransporter family | 12 | 8 | 7 | 0 | 0 | 1 |
| SLC6 | Sodium- and chloride-dependent neurotransmitter transporter family | 20 | 13 | 8 | 4 | 0 | 1 |
| SLC7 | Cationic amino acid transporter/ glycoprotein-associated family | 14 | 10 | 6 | 0 | 0 | 4 |
| SLC8 | $Na^+$–$Ca^{2+}$ exchanger family | 4 | 4 | 1 | 3 | | |
| SLC9 | $Na^+$–$H^+$ exchanger family | 13 | 7 | 5 | 1 | | |
| SLC10 | Sodium bile salt cotransport family | 7 | 4 | 4 | 0 | | |
| SLC11 | Proton-coupled metal ion transporter family | 2 | 1 | 1 | 0 | | |
| SLC12 | Electroneutral cation-coupled Cl cotransporter family | 9 | 8 | 5 | 1 | | |
| SLC13 | Human $Na^+$-sulfate–carboxylate cotransporter family | 5 | 4 | 3 | 0 | | |
| SLC14 | Urea transporter family | 8 | 1 | 1 | 0 | | |
| SLC15 | Proton oligopeptide cotransporter family | 4 | 3 | 2 | 1 | | |
| SLC16 | Monocarboxylate transporter family | 14 | 10 | 7 | 2 | | |
| SLC17 | Vesicular glutamate transporter family | 9 | 5 | 3 | 2 | | |
| SLC18 | Vesicular amine transporter family | 4 | 3 | 2 | 1 | | |
| SLC19 | Folate/thiamine transporter family | 3 | 3 | 2 | 0 | | |
| SLC20 | Type III $Na^+$-phosphate cotransporter family | 2 | 2 | 1 | 1 | 0 | 0 |
| SLC21 | Organic anion transporting family | 11 | 11 | 11 | 0 | 0 | 0 |
| SLC22 | Organic cation/anion/zwitterion transporter family | 23 | 5 | 4 | 1 | 0 | 0 |
| SLC23 | $Na^+$-dependent ascorbic acid transporter family | 4 | 2 | 2 | 0 | 0 | 0 |
| SLC24 | $Na^+$/($Ca^{2+}$–$K^+$) exchanger family | 5 | 4 | 3 | 1 | 0 | 0 |
| SLC25 | Mitochondrial carrier family | 53 | 35 | 26 | 8 | 0 | 1 |
| SLC26 | Multifunctional anion exchanger family | 11 | 8 | 7 | 0 | 0 | 1 |
| SLC27 | Fatty acid transport protein family | 6 | 4 | 2 | 2 | 0 | 0 |
| SLC28 | $Na^+$-coupled nucleoside transport family | 3 | 0 | 0 | 0 | 0 | 0 |
| SLC29 | Facilitative nucleoside transporter family | 4 | 3 | 2 | 0 | 0 | 1 |
| SLC30 | Zinc efflux family | 10 | 9 | 8 | 1 | 0 | 0 |
| SLC31 | Copper transporter family | 2 | 2 | 2 | 0 | 0 | 0 |
| SLC32 | Vesicular inhibitory amino acid transporter family | 1 | 1 | 1 | 0 | 0 | 0 |
| SLC33 | Acetyl-CoA transporter family | 1 | 1 | 1 | 0 | 0 | 0 |

Table 1. Continued

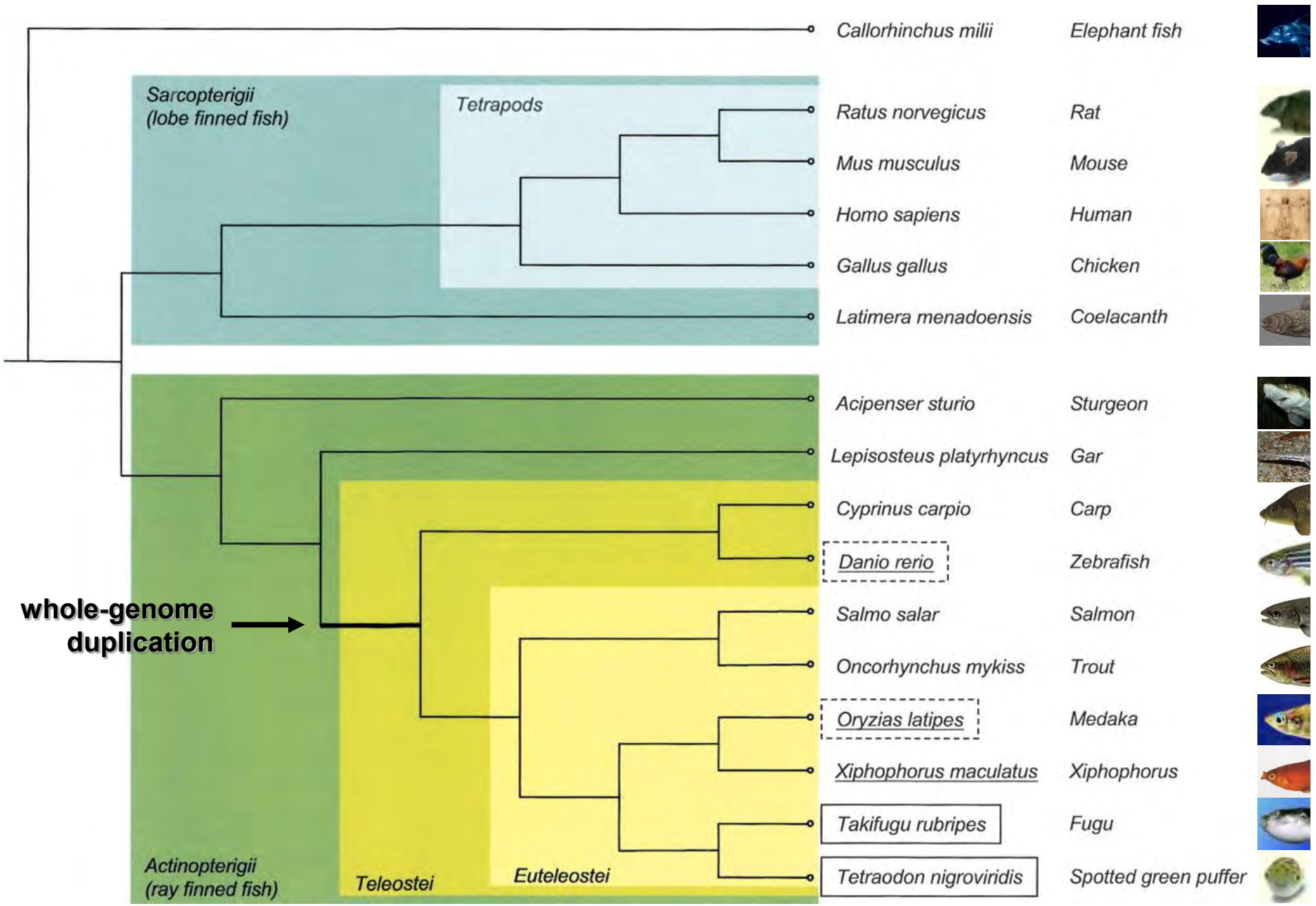
| Family | Name | Human Total genes | Zebrafish Total genes | Zebrafish Non-duplicated genes | Zebrafish Duplicated genes designated as 'a' and 'b' | Zebrafish Duplicated genes designated as 'tandem duplicate' | Zebrafish Possibly duplicated genes and/or genes not obviously designated |
|---|---|---|---|---|---|---|---|
| SLC34 | Type II $Na^+$-phosphate cotransporter family | 3 | 2 | 0 | 1 | 0 | 1 |
| SLC35 | Nucleoside-sugar transporter family | 30 | 19 | 16 | 3 | 0 | 0 |
| SLC36 | Proton-coupled amino acid transporter family | 4 | 1 | 1 | 0 | 0 | 0 |
| SLC37 | Sugar-phosphate/phosphate exchanger family | 4 | 3 | 2 | 1 | 0 | 0 |
| SLC38 | System A & N sodium-coupled neutral amino acid transporter family | 11 | 8 | 6 | 1 | 0 | 1 |
| SLC39 | Metal ion transporter family | 14 | 10 | 10 | 0 | 0 | 0 |
| | | 1 | 1 | 1 | 0 | 0 | 0 |
| | | 3 | 1 | 1 | 0 | 0 | 0 |
| | | 3 | 6 | 5 | 0 | 0 | 1 |
| | | 3 | 3 | 0 | 3 | 0 | 0 |
| | | 5 | 4 | 2 | 2 | 0 | 0 |
| | | 4 | 4 | 4 | 0 | 0 | 0 |
| | | 3 | 2 | 2 | 0 | 0 | 0 |
| | | 2 | 2 | 2 | 0 | 0 | 0 |
| | | 1 | 1 | 0 | 1 | 0 | 0 |
| | | 4 | 4 | 3 | 0 | 0 | 1 |
| | | 1 | 1 | 1 | 0 | 0 | 0 |
| | | 2 | 1 | 1 | 0 | 0 | 0 |
| | | 3 | 2 | 2 | 0 | 0 | 0 |
| Total | | 398 | 275 | 201 | 51 | 2 | 21 |

The total number of 'official members' (i.e. the members for human and zebrafish. The number of zebrafish non-duplicated, duplicated and possibly duplicated/not obviously designated genes is also shown. Data have been obtained by GenBank database search on the Reference Zv9 Primary Assembly (July 2013).

**52 families**
**275 genes**

# The SLC family series in zebrafish (*Danio rerio*)

Adapted from:
Romano et al., *J. Physiol.* 592:881 (2014)

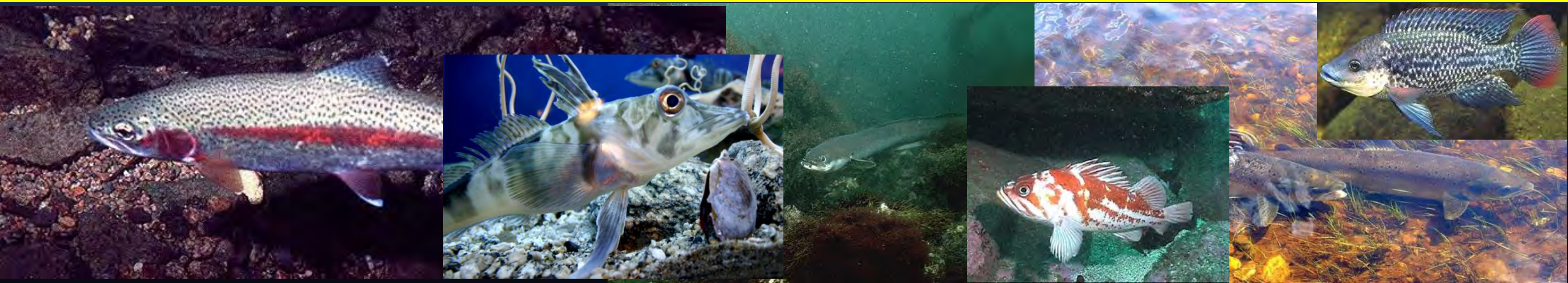



**Whole genome duplication event(s)**

# Genome size of ‘model’ vertebrates’

| | Haploid DNA content (pg) | Haploid genome size (Mb) | No. of chromosomes (*n*) |
|---|---|---|---|
| *Mammals* | | | |
| Human (*Homo sapiens*) | 3.5 | 3000 | 23 |
| Mouse (*Mus musculus*) | 3.5 | 3000 | 20 |
| Rat (*Rattus norvegicus*) | 3.5 | 3000 | 21 |
| *Bird* | | | |
| Chicken (*Gallus gallus*) | 1.25 | 1200 | 39 |
| *Amphibians* | | | |
| *Xenopus laevis* | 3.2 | 3100 | 18 |
| *Xenopus tropicalis* | 1.78 | 1700 | 10 |
| *Fish* | | | |
| Zebrafish (*Danio rerio*) | 1.8 | 1700 | 25 |
| Medaka (*Oryzias latipes*) | 1.1 | 1100 | 24 |
| Fugu (*Fugu rubripes*) | | 400 | 22 |

The chicken karyotype includes 30 microchromosomes in addition to nine macrochromosomes. *X. laevis* is a tetraploid whereas *X. tropicalis* is a diploid. The DNA content of Fugu has not been determined. References: chicken [44]; *Xenopus* [45]; zebrafish [46]; medaka [47] and Fugu [1,48].



# The SLC family series in teleost fish

***"Hot" in fish physiology and pathophysiology***

- ***Digestive/absorptive system***
- ***Sensory system/brain***
- ***Muscle and body skeleton***
- ***Buoyancy and swim-bladder***
- ***Osmoregulation***
- ***Respiration and excretion***
- ***Neuroendocrine system***
- ***Immune system***
- ***Skin and pigmentation***

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***"Hot" in fish physiology and pathophysiology***

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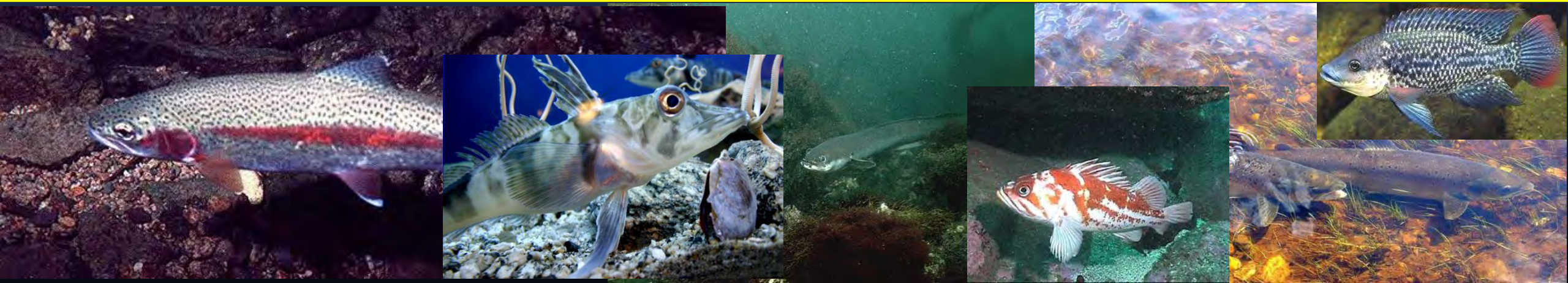
# Peptide transport(ers) in teleost fish



# SLC15:
# the proton oligopeptide cotransporter family

| Human Gene Name | Protein Name | Predominant Substrates | Transport type / Coupling ions | Tissue distribution and cellular / subcellular expression | Human gene locus | Splice variants and their specific features |
|---|---|---|---|---|---|---|
| *SLC15A1* | PEPT1 | Di- and tripeptides, protons | Cotransporter / $H^+$ | Intestine, kidney apical, lysosomal membrane | 13q33-q34 | hPEPT1-RF shift of pH sensitivity profile |
| *SLC15A2* | PEPT2 | Di- and tripeptides, protons | Cotransporter / $H^+$ | Kidney, lung, brain, mammary gland, bronchial epithelium | 3q13.3-q21 | |
| *SLC15A3* | PHT2 hPTR3 | Histidine, di- and tripeptides, protons | Cotransporter / $H^+$ | Lung, spleen, thymus (faintly in brain, liver, adrenal gland, heart) | 11q12.1 | multiple, features unknown |
| *SLC15A4* | PHT1 PTR4 | Histidine, di- and tripeptides, protons | Cotransporter / $H^+$ | Brain, retina, placenta | 12q24.32 | multiple, features unknown |

The HUGO Solute Carrier Family Series

Adapted from:
Daniel & Kottra, Pflügers Arch. 447:610-618 (2004)

400 dipeptides
8000 tripeptides

# Not all di- and tripeptides are good substrates!

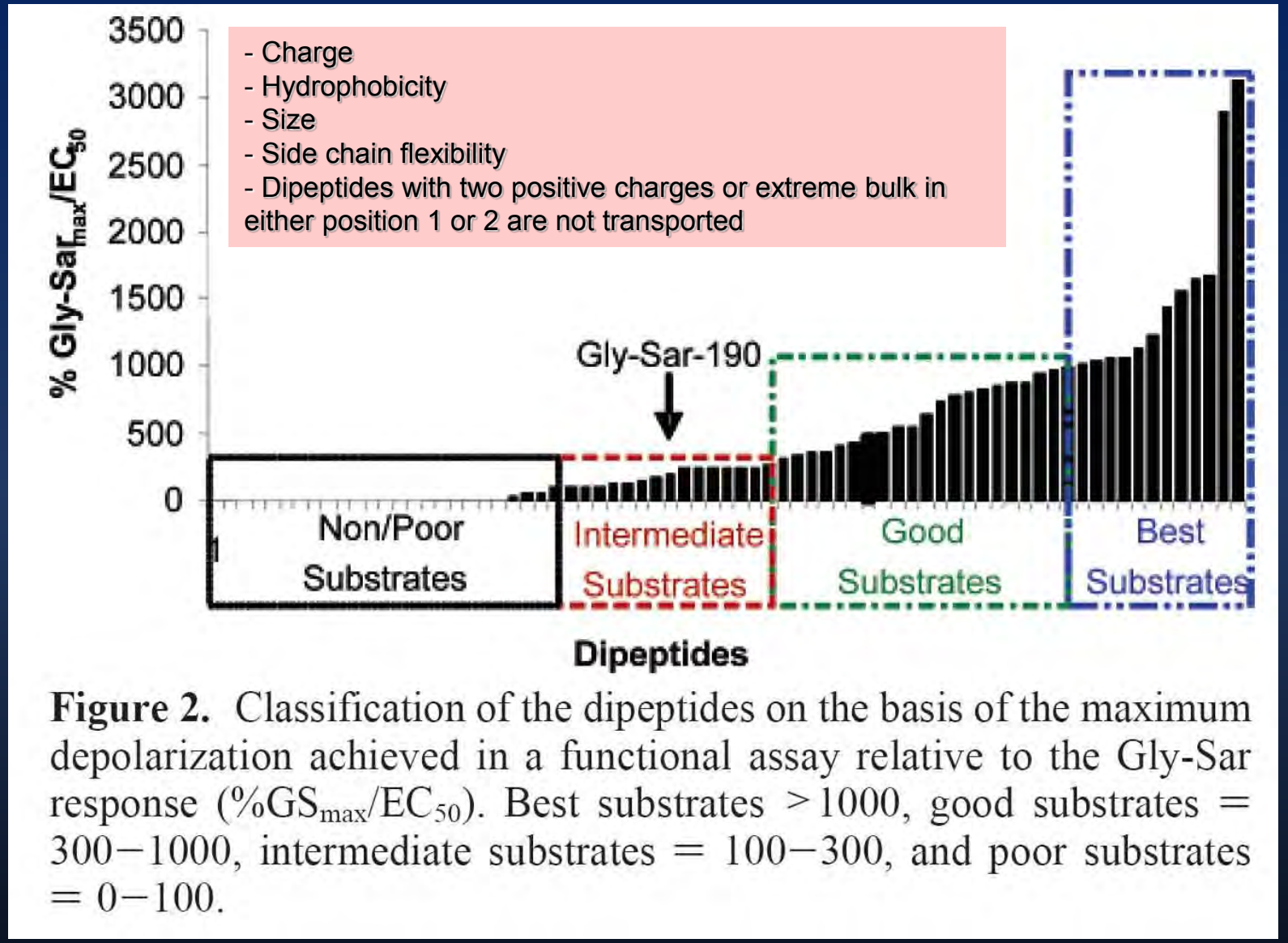



**Figure 2.** Classification of the dipeptides on the basis of the maximum depolarization achieved in a functional assay relative to the Gly-Sar response (%GS$_{max}$/EC$_{50}$). Best substrates >1000, good substrates = 300−1000, intermediate substrates = 100−300, and poor substrates = 0−100.

Adapted from:
Vig et al., J. Med. Chem. 49:3636-3644 (2006)

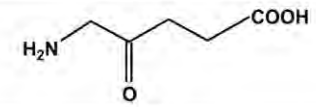
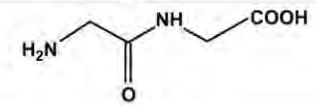
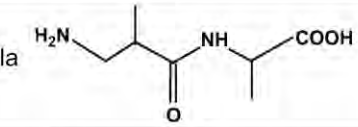
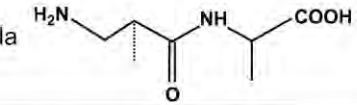
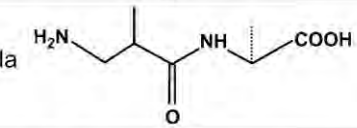
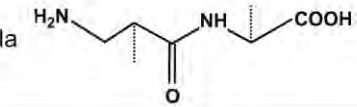
**Table 1.** PEPT1 Activity and Inhibition Data

| no. | name | $EC_{50}$ (mM) mean ± SD | $\%GS_{max}$ mean ± SD | $\%GS_{max}/EC_{50}$ | $IC_{50}$ (mM) | no. | name | $EC_{50}$ (mM) mean ± SD | $\%GS_{max}$ mean ± SD | $\%GS_{max}/EC_{50}$ | $IC_{50}$ (mM) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Ac-Phe-di-iodo-Tyr | NC[a] | NA[b] | | 0.23 | 41 | His-Gly | 0.28 ± 0.03 | 128 ± 28 | 460 | 0.34 |
| 2 | Ac-Phe-Tyr-$NH_2$ | NC | NA | | NA | 42 | His-His | 0.37 ± 0.04 | 132 ± 33 | 350 | 0.40 |
| 3 | Ala-Ala | 0.08 ± 0.01 | 125 ± 19 | 1700 | 0.25 | 43 | His-Trp | 0.19 | 28 | 150 | 0.95 |
| 4 | Ala-Asp | 0.23 ± 0.04 | 124 ± 10 | 540 | 0.45 | 44 | Leu-Leu | 0.08 ± 0.03 | 108 ± 29 | 1400 | 0.17 |
| 5 | Ala-Lys | 0.22 ± 0.04 | 117 ± 16 | 540 | 0.28 | 45 | Lys-Arg | NC | NA | | 7.20 |
| 6 | Ala-Phe | 0.08 ± 0.02 | 135 ± 34 | 1700 | 0.07 | 46 | Lys-Glu | 0.53 ± 0.05 | 121 ± 21 | 230 | 0.82 |
| 7 | Ala-Trp | 0.08[c] | 64 ± 4 | 830 | 0.26 | 47 | Lys-Gly | 0.32 ± 0.06 | 130 ± 27 | 410 | 0.38 |
| 8 | Ala-Tyr | 0.06 ± 0.01 | 90 ± 23 | 1600 | 0.17 | 48 | Lys-Lys | NC | NA | | 10.9 |
| 9 | Arg-Arg | NC | NA | | 7.31 | 49 | Lys-Pro | 0.19 ± 0.03 | 138 ± 8 | 720 | 0.39 |
| 10 | Arg-Gly | 0.27 ± 0.06 | 136 ± 32 | 500 | 0.39 | 50 | Lys-Trp | NC | NA | | 0.66 |
| 11 | Arg-Lys | NC | NA | | 8.11 | 51 | Lys-Val | 0.14 ± 0.06 | 132 ± 6 | 960 | 0.25 |
| 12 | Asp-Asp | 0.99 | 99 ±11 | 100 | 0.63 | 52 | Orn-Orn | NC | NA | | NA |
| 13 | Asp-Gly | 0.44 | 107 ± 15 | 240 | 0.81 | 53 | Phe-Ala | 0.11 ± 0.05 | 108 ± 20 | 1000 | 0.07 |
| 14 | Asp-Trp | 0.47 ± 0.36 | 22 ± 5 | 46 | 1.31 | 54 | Phe-Ala-$NH_2$ | 0.85 | 39 | 50 | 2.99 |
| 15 | Asp-Val | 0.69 ± 0.28 | 78 ± 4 | 110 | 0.31 | 55 | Phe-Gly | 0.11 ± 0.00 | 120 ± 11 | 1100 | 0.17 |
| 16 | Gln-Gln | 0.10 ± 0.02 | 77 ±12 | 790 | 0.15 | 56 | Phe-Phe | 0.03 ± 0.02 | 105 ± 19 | 3100 | 0.08 |
| 17 | Gln-Glu | 0.42 ± 0.08 | 97 ± 4 | 230 | 0.51 | 57 | Phe-Tyr | 0.03 ± 0.01 | 78 ± 14 | 2900 | 0.02 |
| 18 | Glu-Glu | 1.00 ± 0.17 | 111 ± 3 | 110 | 0.62 | 58 | Pro-Asp | >5 | 104 ± 30 | | 9.16 |
| 19 | Glu-Gly | 0.51 ± 0.05 | 122 ± 10 | 240 | 0.39 | 59 | Pro-Glu | >5 | 65 ± 14 | | 12.3 |
| 20 | Glu-Lys | 0.31 ± 0.15 | 83 ± 12 | 270 | 0.72 | 60 | Pro-Gly | NC | 42 ± 27 | | >16 |
| 21 | Gly | NC | NA | NC | NC | 61 | Pro-Leu | 0.25 ± 0.06 | 76 ± 4 | 300 | 0.62 |
| 22 | Gly-Arg | 0.52 ± 0.05 | 55 ± 20 | 100 | 1.82 | 62 | Pro-Lys | NC | NA | | >16 |
| 23 | Gly-Asp | 0.55 ± 0.28 | 124 ± 16 | 230 | 0.38 | 63 | Pro-Pro | 0.70 ± 0.16 | 116 ± 31 | 170 | 0.80 |
| 24 | Gly-Glu | 1.10 ± 0.41 | 113 ± 12 | 100 | 0.65 | 64 | Pro-Ser | 1.6 ± 0.10 | 31 ± 25 | 20 | >16 |
| 25 | Gly-Gly | 0.48 ± 0.18 | 112 ± 18 | 230 | 0.82 | 65 | Ser-Ser | 0.14 ± 0.02 | 108 ± 9 | 770 | 0.13 |
| 26 | Gly-Gly-Gly | 0.58 ± 0.06 | 109 ± 10 | 190 | 1.07 | 66 | Trp-Ala | 0.10 ± 0.02 | 98 ± 18 | 1000 | 0.26 |
| 27 | Gly-Gly-Gly-Gly | NC | 15 | | NA | 67 | Trp-Gly | 0.26 ± 0.05 | 92 ± 10 | 350 | 0.73 |
| 28 | Gly-Gly-Gly-$NH_2$ | | | | NA | 68 | Trp-Trp | NC | NA | | 0.25 |
| 29 | Gly-His | 0.40 ± 0.06 | 128 ± 31 | 320 | 0.81 | 69 | Trp-Tyr | NC | NA | | 0.08 |
| 30 | Gly-Leu | 0.17 ± 0.11 | 103 ± 18 | 620 | 0.07 | 70 | Trp-Val | 0.05 ±0.02 | 58 ± 5 | 1100 | 0.09 |
| 31 | Gly-Leu-Gly | 0.21 ± 0.05 | 113 ± 29 | 530 | 0.24 | 71 | Tyr-Ala | 0.10 ±0.05 | 102 ± 21 | 1110 | 0.11 |
| 32 | Gly-Leu-Phe | 0.28 ± 0.07 | 83 ± 7 | 290 | 0.98 | 72 | Tyr-Gly | 0.24 ±0.06 | 99 ± 18 | 420 | 0.33 |
| 33 | Gly-Lys | 0.75 ± 0.37 | 78 ± 5 | 100 | 1.25 | 73 | Tyr-Gly-$NH_2$ | NC | NA | | NA |
| 34 | Gly-Phe | 0.13 ± 0.01 | 111 ± 17 | 830 | 0.17 | 74 | Tyr-Tic-$NH_2$ | NC | NA | | NA |
| 35 | Gly-Phe-$NH_2$ | NC | | | NA | 75 | Tyr-Trp | NC | 46 ± 8 | | 0.10 |
| 36 | Gly-Pro | 0.13 ± 0.03 | 111 ± 27 | 870 | 0.33 | 76 | Tyr-Tyr | 0.06 ± 0.01 | 69 ± 12 | 1200 | 0.06 |
| 37 | Gly-Sar | 0.54 ± 0.20 | 101 ± 7 | 190 | 1.16 | 77 | Tyr-Tyr-$NH_2$ | NC | NA | | 9.11 |
| 38 | Gly-Trp | 0.33 ± 0.08 | 23 ± 7 | 70 | 0.52 | 78 | Val | NC | NA | NC | NC |
| 39 | Gly-Tyr | 0.14 ± 0.01 | 125 ± 27 | 870 | 0.12 | 79 | Val-Trp | 0.04 ± 0.01 | 37 ± 21 | 950 | 0.10 |
| 40 | Gly-Tyr-$NH_2$ | NC | NA | | NA | 80 | Val-Val | 0.07 ± 0.02 | 74 ± 14 | 1000 | 0.21 |
| | | | | | | 81 | Val-Val-Val | 0.21 ± 0.04 | 99 ± 28 | 480 | 0.23 |

[a] NA = No activity. [b] NC = Not calculable. [c] At places, the standard deviation is not provided. This may be due to either compounds causing insufficient activation of PEPT1 for parameter calculation or not enough repeats ($n = 2$) for few of the compounds.

# The key structural and conformational elements in PEPT1 substrates and how they affect substrate affinity and electrogenic transport

This series of model compounds has been analyzed with respect to substrate affinity and electrogenic transport under identical experimental conditions in *Pichia pastoris* cells and *Xenopus* oocytes expressing PEPT1. Apparent substrate affinities are derived from competition experiments with the model compounds in P. pastoris cells with a radioactive dipeptide serving as substrate. Inward currents generated by the compounds in *Xenopus* oocytes expressing PEPT1, determined by the two-voltage-clamp technique, are used to express the maximal transport rate. The test compounds have been applied under substrate saturation conditions and maximal transport currents are expressed as $I_{max}$ in percent of that elicited by 10 mM Gly-L-Gln serving as a control in the same batch of oocytes. The comparison shows the most critical structural elements in substrates such as the intramolecular distance between the centers of the amino- and carboxy-terminal head groups and the central carbonyl function. Moreover, the stereoselective recognition of substrate side chains is demonstrated on basis of alanyl-peptides with D- and L-residues at different positions in the dipeptide.

| compound | structure | apparent affinity [mM] | transport currents ($I_{max}$ % of control) |
|---|---|---|---|
| 4-aminobutyric acid |  | > 50 | 0 |
| 5-aminopentanoic acid |  | 1.14 ± 0.06 | 100 |
| 5-amino-4-oxo-pentanoic acid |  | 0.27 ± 0.04 | 100 |
| Gly-Gly |  | 0.20 ± 0.02 | 100 |
| L-Ala-L-Ala |  | 0.16 ± 0.03 | 100 |
| D-Ala-L-Ala |  | 0.80 ± 0.06 | 70 |
| L-Ala-D-Ala |  | 6.12 ± 0.34 | 30 |
| D-Ala-D-Ala |  | > 25 | 0 |

# The key structural and conformational elements in PEPT1 substrates and how they affect substrate affinity and electrogenic transport

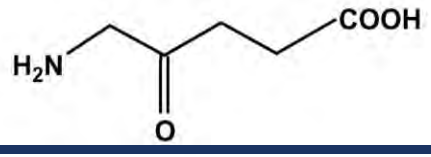
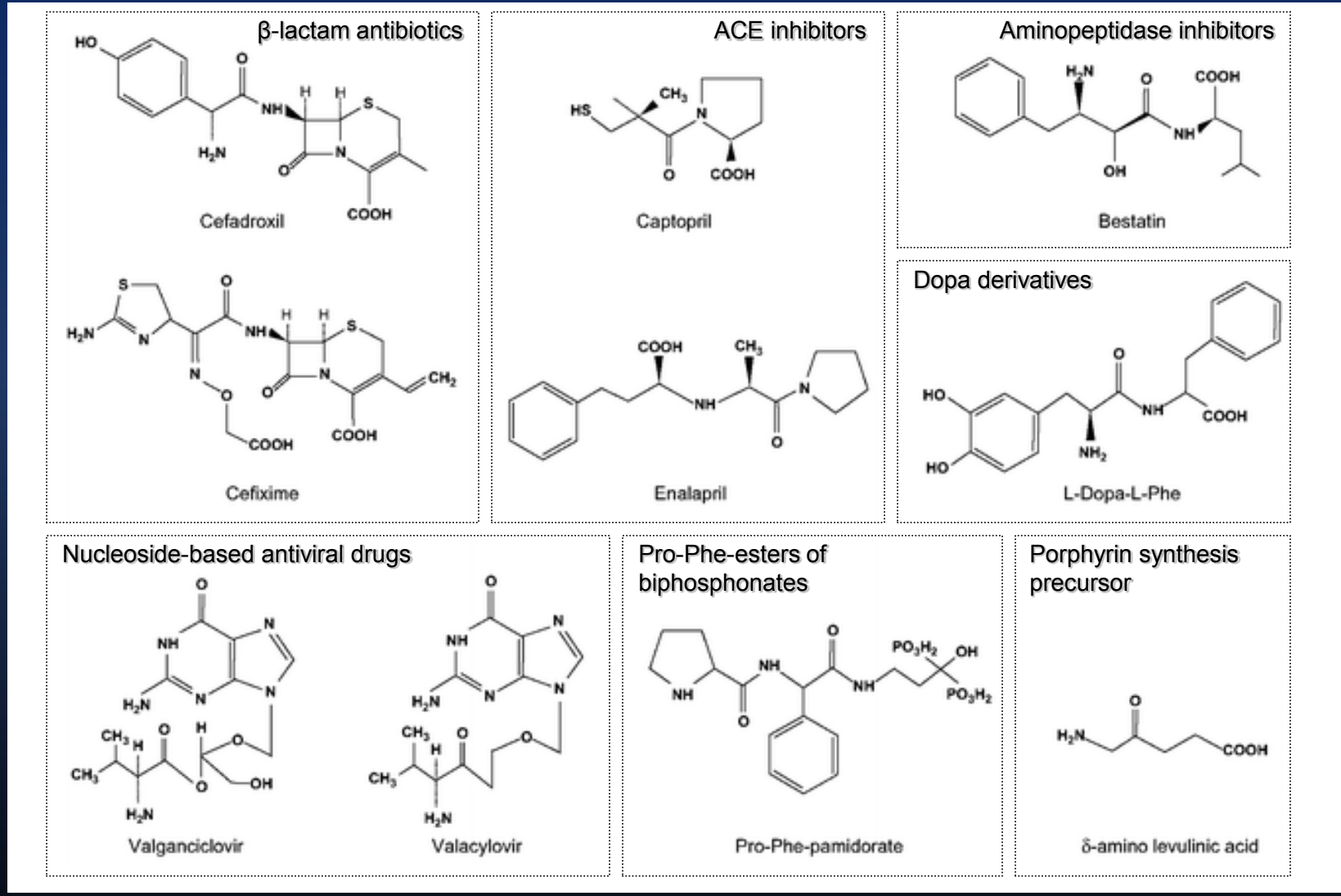
| compound | structure | apparent affinity [mM] | transport currents ($I_{max}$ % of control) |
|---|---|---|---|
| 5-amino-4-oxo-pentanoic acid |  | 0.27 ± 0.04 | 100 |

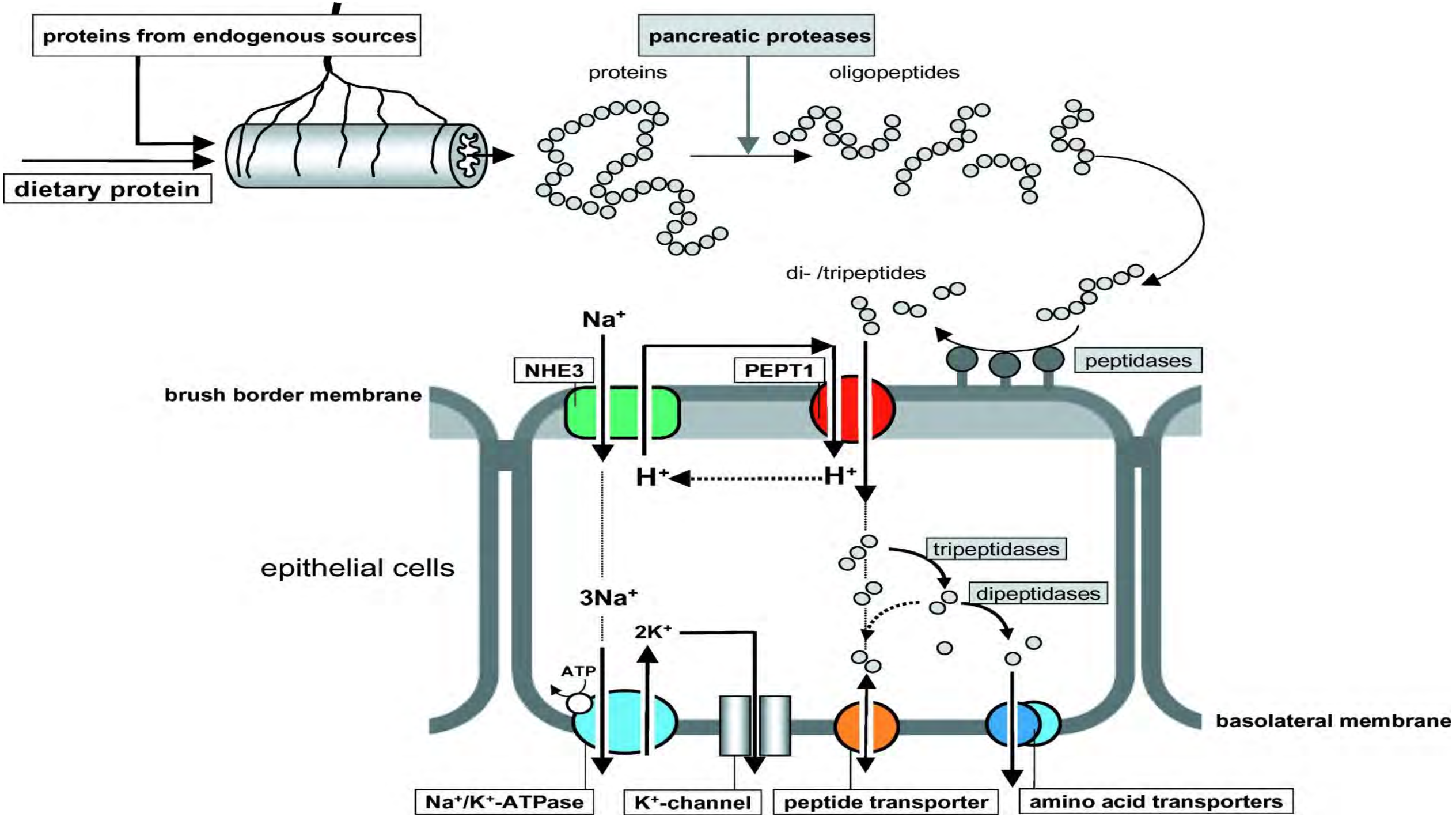


**Figure 1.** The 3D structure of **14** and the three-point recognition model for PEPT1 (D = Donor, A = Acceptor and $e^-$ = high electron density).

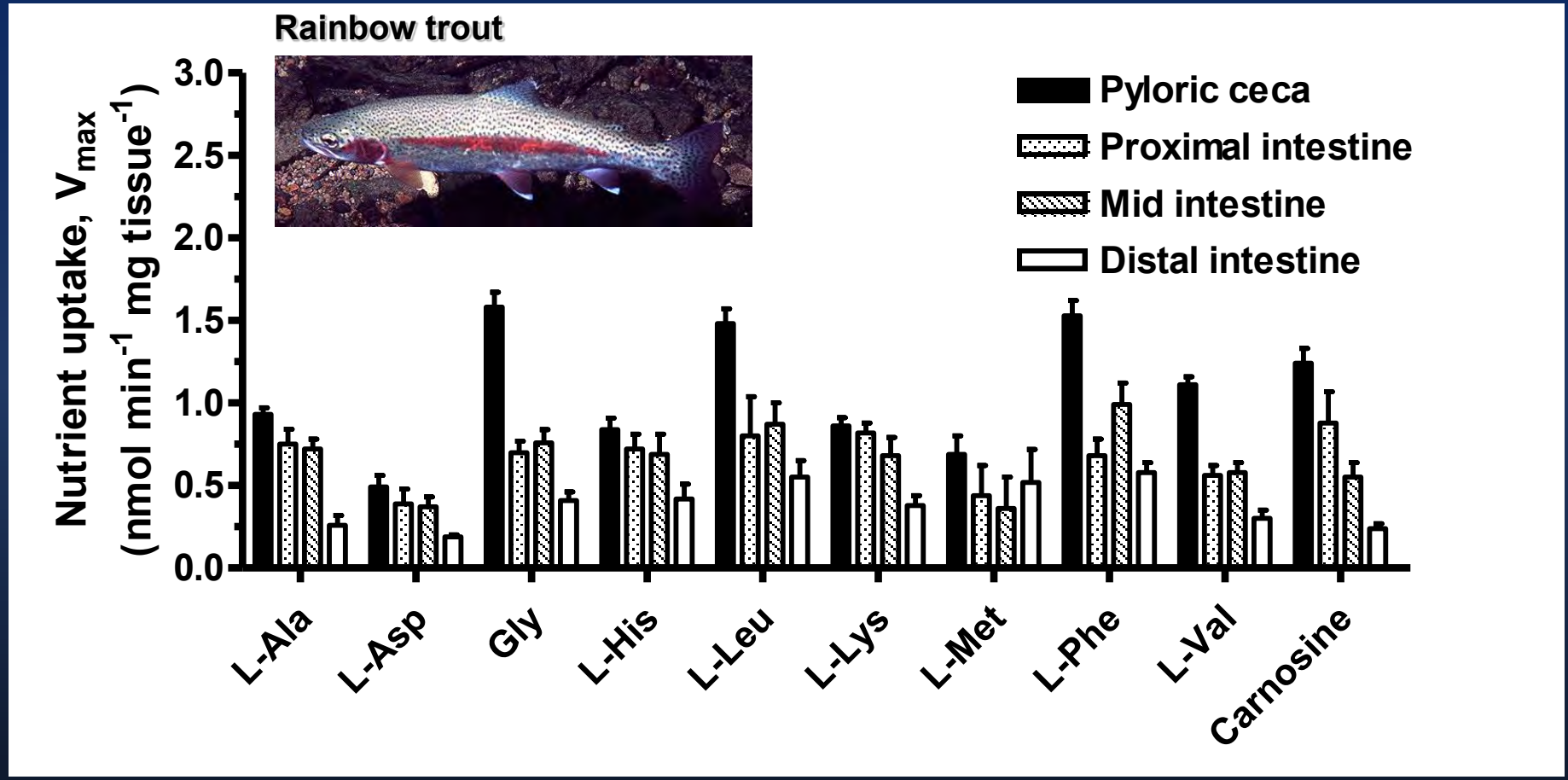
Adapted from:
Gebauer et al., J. Med. Chem. 46:5725-5734 (2003)

# Molecular structures of selected pharmacologically important compounds that serve as substrates of PEPT1



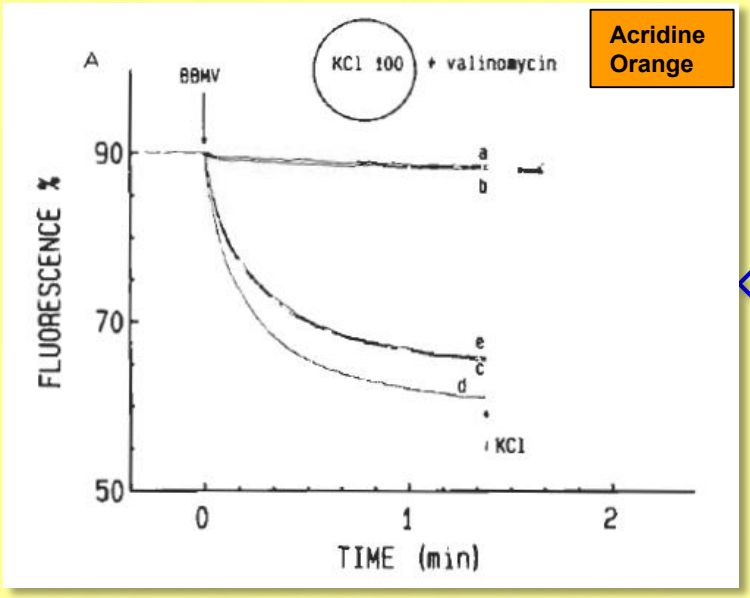

proteins from endogenous sources
dietary protein
pancreatic proteases
proteins
oligopeptides
di- /tripeptides
peptidases
Na+
NHE3
PEPT1
brush border membrane
H+
H+
tripeptidases
dipeptidases
epithelial cells
3Na+
2K+
ATP
basolateral membrane
Na+/K+-ATPase
K+-channel
peptide transporter
amino acid transporters

# Uptake of nine amino acids and the dipeptide β-Ala-L-His (carnosine) in rainbow trout gut




Data adapted from:
Buddington & Diamond, Am. J. Physiol. 252:G65 (1987)

# Carrier-mediated transport of peptides in fish intestine



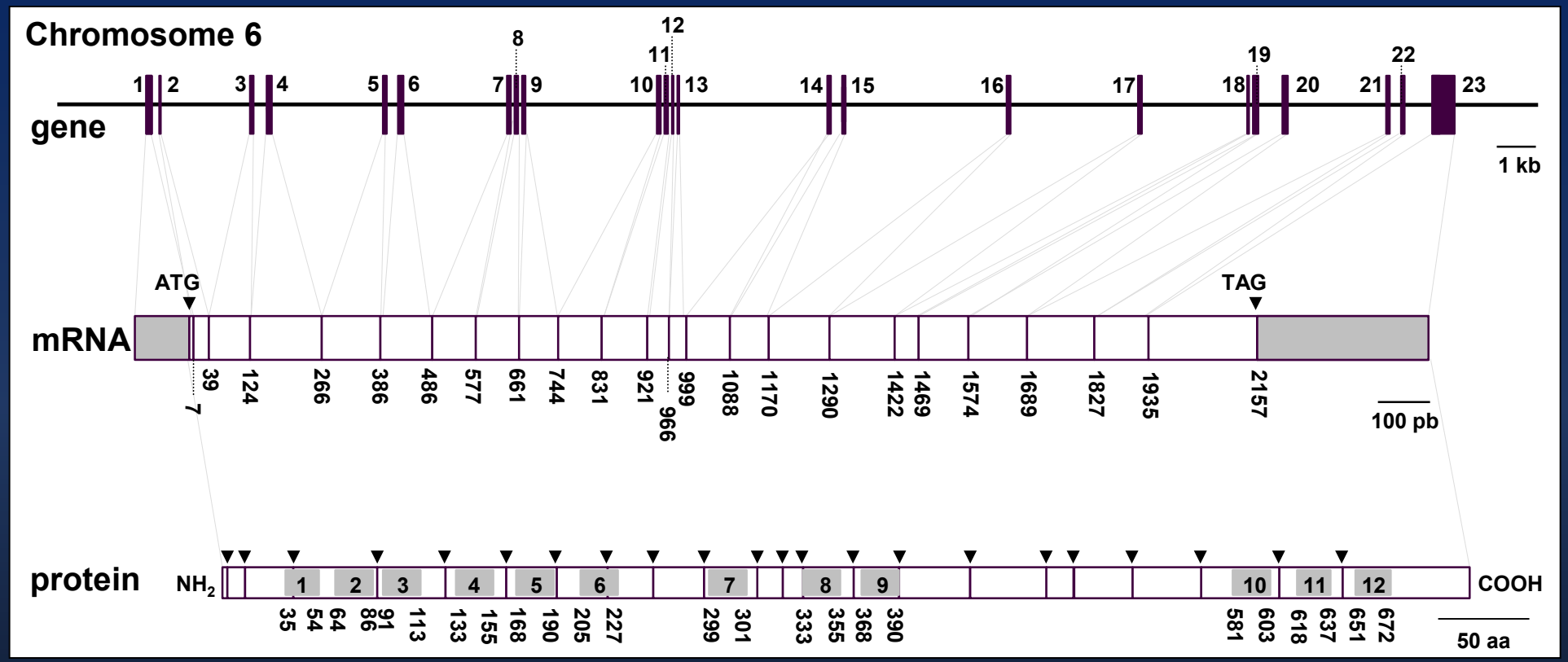

Acridine Orange

| Species | Tissue (Method) | Substrate | $K_m$ (mM) | Reference |
|---|---|---|---|---|
| European eel (*A. anguilla*) | Whole intestine (BBMV) | Gly-L-Pro | 1.27 ± 0.01 | Maffia et al. (1997) *Am. J. Physiol.* 272:R217 |
| | | | 1.32 ± 0.10 | Maffia et al. (1997) *Am. J. Physiol.* 272:R217 |
| | | | 1.04 ± 0.31 | Verri et al. (2000) *J. Exp. Biol.* 203:2991 |
| | | | 1.43 ± 0.53 | Verri et al. (2008) *Aquacult. Nutr.* 14:341 |
| | | | 1.68 ± 1.01 | Verri et al. (2008) *Aquacult. Nutr.* 14:341 |
| | | D-Phe-L-Ala | 0.74 ± 0.16 | Verri et al. (2000) *J. Exp. Biol.* 203:2991 |
| | | | 1.19 ± 0.52 | Verri et al. (2000) *J. Exp. Biol.* 203:2991 |
| | | Gly-Gly | 12.36 ± 3.13 | Verri et al. (1992) *Biochim. Biophys. Acta* 1110:123 |
| | | | 1.81 ± 0.49 | Verri et al. (2000) *J. Exp. Biol.* 203:2991 |
| | | | 1.59 ± 0.40 | Verri et al. (2008) *Aquacult. Nutr.* 14:341 |
| | | | 2.49 ± 0.84 | Verri et al. (2008) *Aquacult. Nutr.* 14:341 |
| | | Gly-L-Ala | 0.97 ± 0.42 | Verri et al. (2000) *J. Exp. Biol.* 203:2991 |
| | | Gly-L-Asn | 2.59 ± 0.73 | Verri et al. (2000) *J. Exp. Biol.* 203:2991 |
| | | Gly-Sar | 1.75 ± 0.47 | Verri et al. (2000) *J. Exp. Biol.* 203:2991 |
| | | L-Pro-Gly | 0.87 ± 0.36 | Verri et al. (2000) *J. Exp. Biol.* 203:2991 |
| Mozambique tilapia (*O. mossambicus*) | Whole intestine (BBMV) | Gly-L-Phe | 9.8 ± 3.5 | Reshkin & Ahearn (1991) *Am. J. Physiol.* 260:R563 |
| | Upper one-half intestine (BBMV) | Gly-Sar | 0.56 ± 0.08 | Thamotharan et al. (1996) *Am. J. Physiol.* 270:R939 |
| Antarctic icefish (*C. hamatus*) | Whole intestine (BBMV) | Gly-L-Pro | 0.806 ± 0.161 | Maffia et al. (2003) *J. Exp. Biol.* 206:705 |
| Mozambique tilapia (*O. mossambicus*) | Upper one-half intestine (BBMV) | Gly-Sar | 13.27 ± 3.80 | Thamotharan et al. (1996) *Am. J. Physiol.* 270:R948 |
| Atlantic salmon (*S. salar*) | Pyloric ceca (everted sleeves) | Gly-L-Pro | 0.5 ± 0.4 | Bakke-McKellep et al. (2000) *Fish Physiol. Biochem.* 22:33 |
| | Mid intestine (everted sleeves) | | 1.5 ± 0.4 | Bakke-McKellep et al. (2000) *Fish Physiol. Biochem.* 22:33 |
| | Distal intestine (everted sleeves) | | ND | Bakke-McKellep et al. (2000) *Fish Physiol. Biochem.* 22:33 |
| | Proximal intestine (everted sleeves) | Gly-Sar | 8.579 ± 5.327 | Nordrum et al. (2000) *Comp. Biochem. Physiol. B* 125:317 |
| | | | 13.120 ± 6.620 | Nordrum et al. (2000) *Comp. Biochem. Physiol. B* 125:317 |
| | | | 1.370 ± 0.118 | Nordrum et al. (2000) *Comp. Biochem. Physiol. B* 125:317 |
| Rainbow trout (*O. mykiss*) | Proximal intestine (everted sleeves) | Gly-Sar | 9.774 ± 8.736 | Nordrum et al. (2000) *Comp. Biochem. Physiol. B* 125:317 |
| | | | 0.747 ± 0.051 | Nordrum et al. (2000) *Comp. Biochem. Physiol. B* 125:317 |
| | | | 5.270 ± 1.41 | Nordrum et al. (2000) *Comp. Biochem. Physiol. B* 125:317 |

# Peptide transport(ers) in the zebrafish

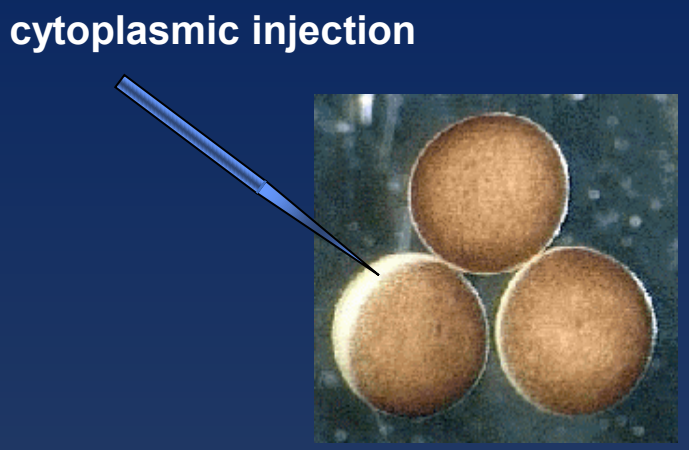
# Zebrafish *slc15a1* (*pept1*)

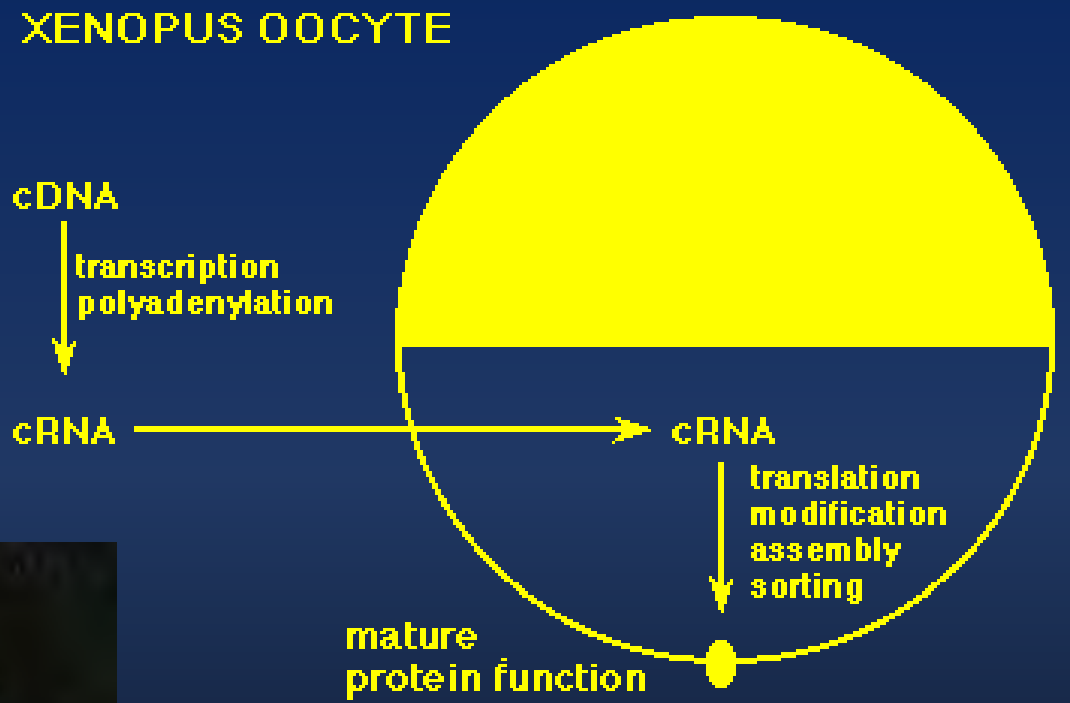
# Zebrafish *slc15a1* (*pept1*): gene, mRNA and predicted protein structure




- *slc15a1* (*pept1*) gene: 37,702 bp
- *slc15a1* (*pept1*) mRNA: 2,746 bp (open reading frame: 2,157 bp)
- Slc15a1 (Pept1) protein: 718 aa (12 transmembrane domains, 6 Asn glycosylation sites, 1 PKA site, 2 PKC sites)

# Expression of membrane transporters in *Xenopus laevis* oocytes




**Analysis of the expressed protein**

- Radioactive tracers
- Two-Electrode Voltage Clamp
- Others

Romero et al., Methods Enzymol. 296:17 (1998)