

Importance of peptide selection in absolute and relative quantitation of drug transporters

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June 3rd, 2018

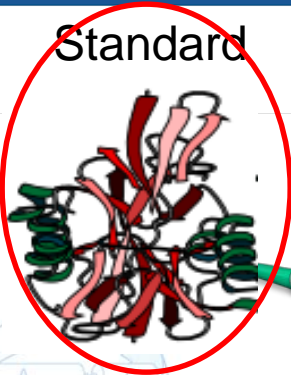
Protein vs peptide based quantitation

Sample

Standard

MDR1

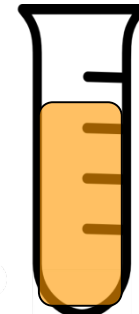
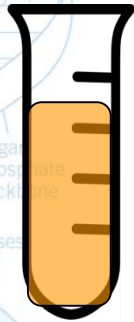
Sample



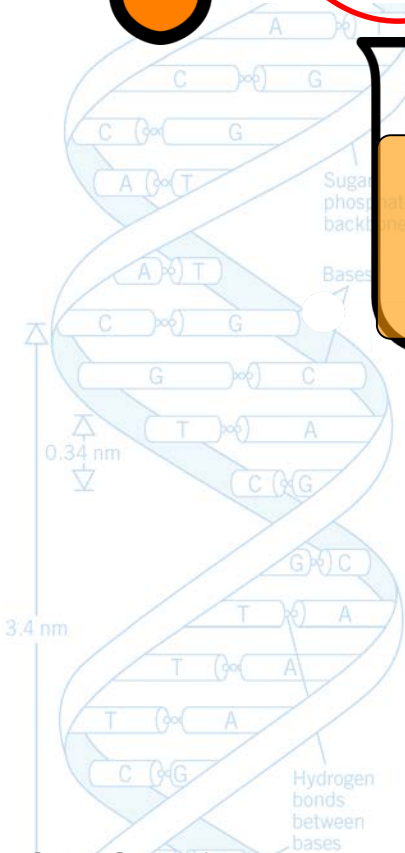
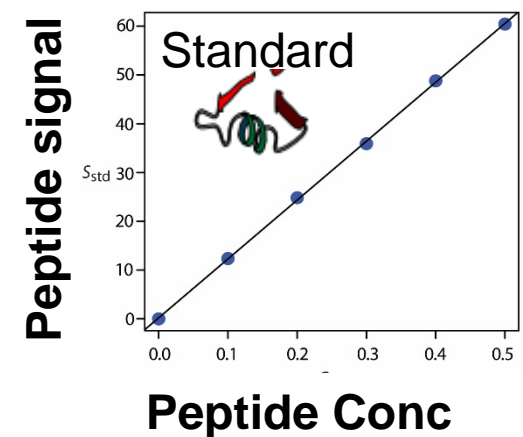
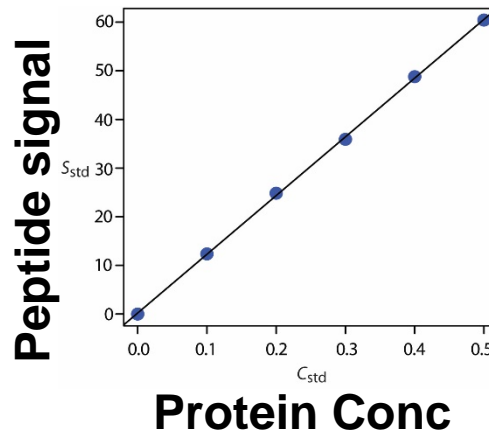
Extraction



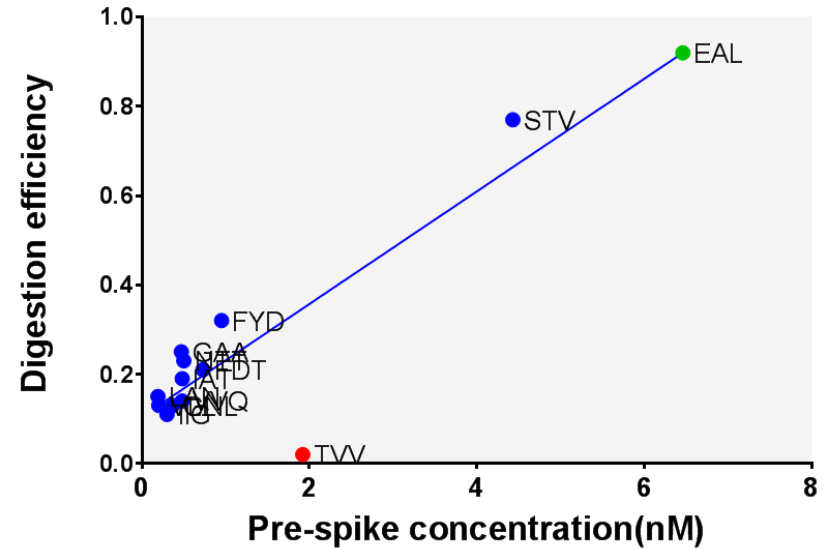
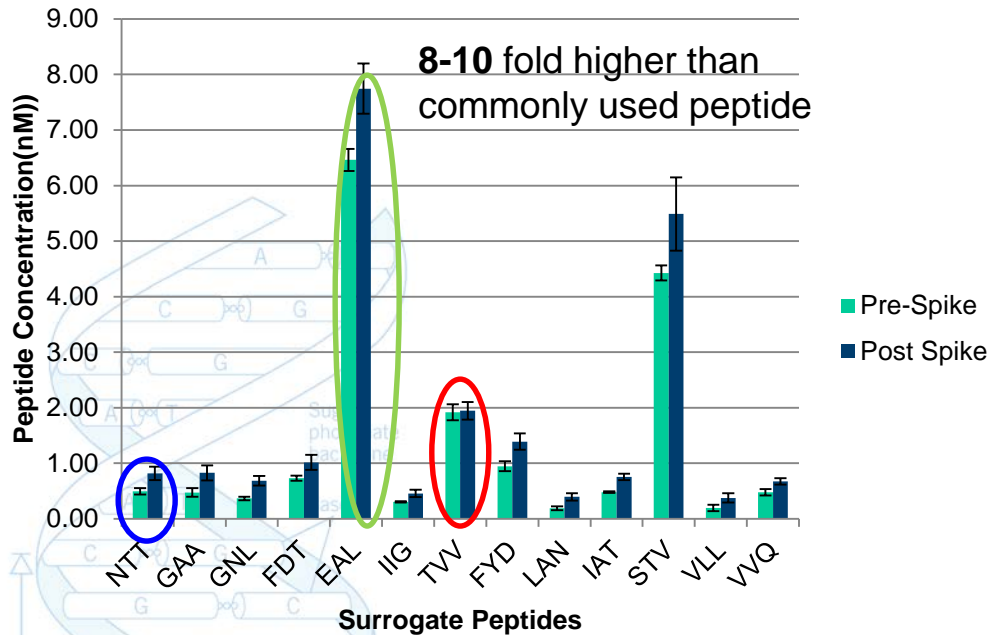
Digestion



Quantitation



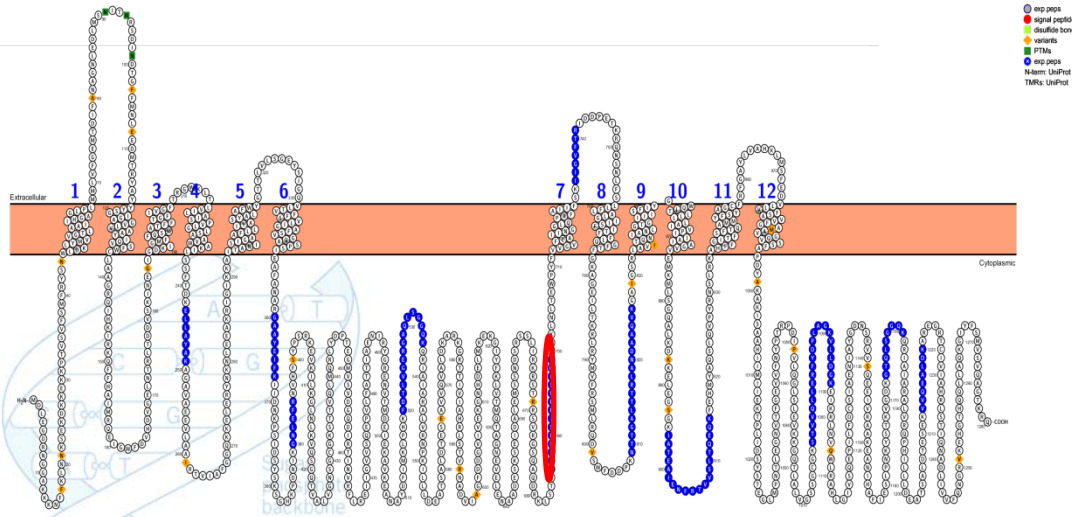
First example from MDR1



$$\text{Digestion efficiency} = \frac{\text{Post Spike} - \text{Pre Spike peptide concentration}}{\text{Spiked in protein concentration}}$$

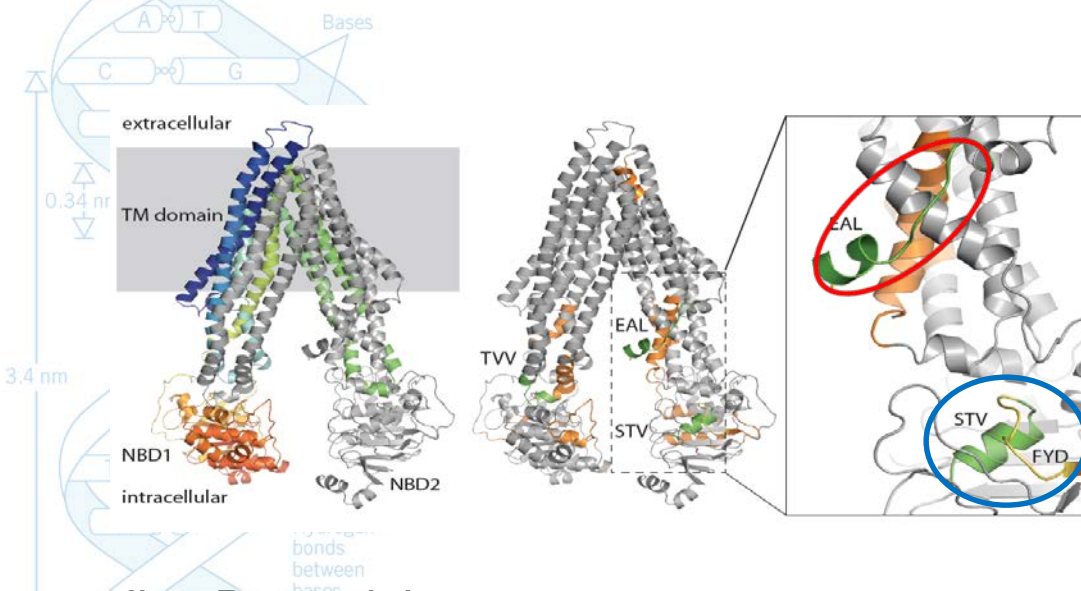
Chen, B. et al, *The AAPS Journal* 2017, epub

Reasons for different digestion efficiency



EALDESIPPVSFWR

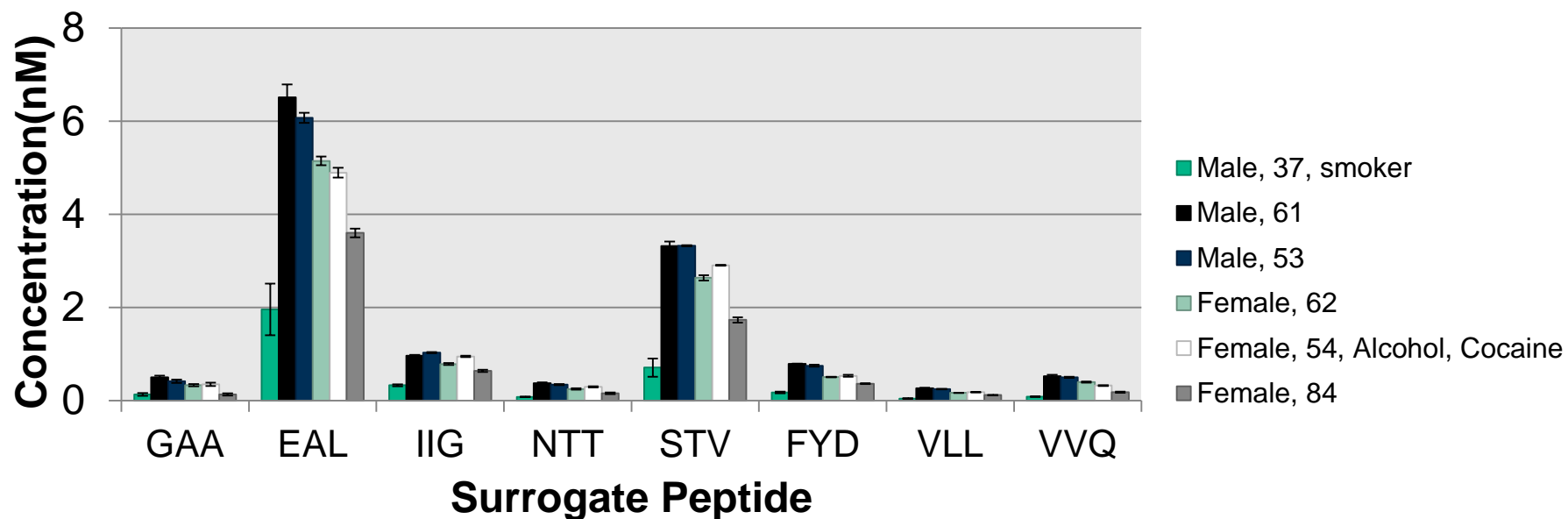
1. “Elbow region” connecting two halves of homo dimer in lower species
2. Not an integral part of large stretches of tertiary structures



STV and FYD

Adjacent to ATP binding sequence more accessible to digestion enzyme

Determination in liver and comparison to literature



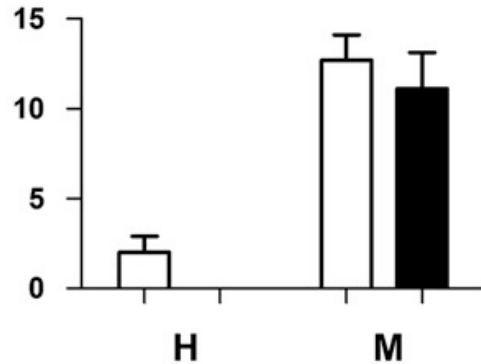
Reference	Ohtsuki et al, 2012 ¹⁴	Prasad et al, 2013 ¹⁵	Peng et al, 2015 ¹²	This study
n=	17	64	141	6
MDR1(NTT)	1.50±0.44	0.4±0.2	0.6±0.2	0.90±0.37
MDR1(IAT)		0.3±0.2		0.77±0.47
MDR1(EAL)				16.78±5.48
Extraction	Ultracentrifugation PM	Millipore kit PM	Millipore kit PM	Millipore kit PM
Digestion enhancement procedure	6M Urea	Extraction buffer II	DOC, dilute/dry	DOC, spin column

Chen, B. et al, *The AAPS Journal* 2017, epub

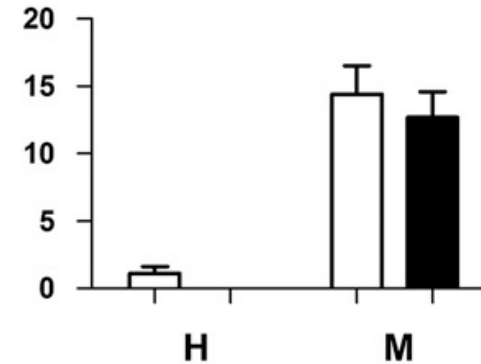
OATPs: comparison of different species

Literature data

OATP1B1/Oatp1b1



OATP1B3/Oatp1b3

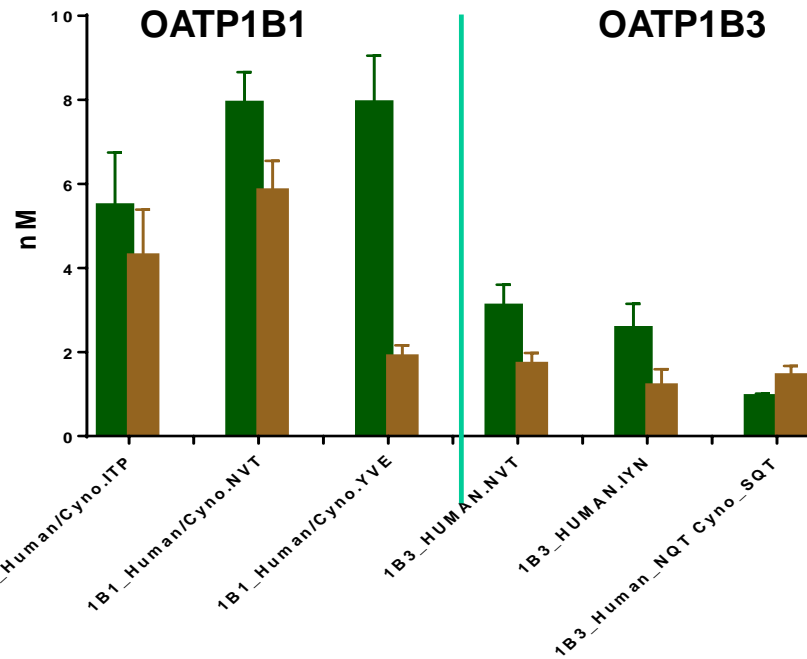


● Hepatocyte
○ Liver

Wang, *et al*, *Drug Metab Dispos* 43:367–374, March 2015

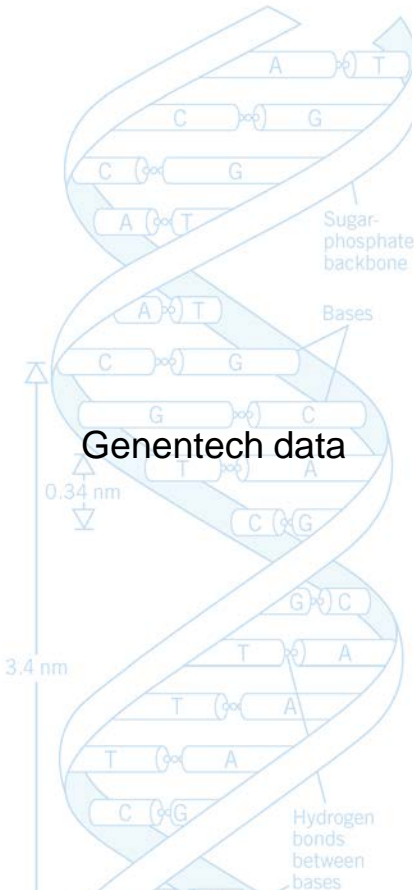
Genentech data

OATP1B1

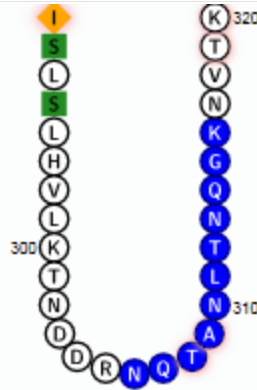
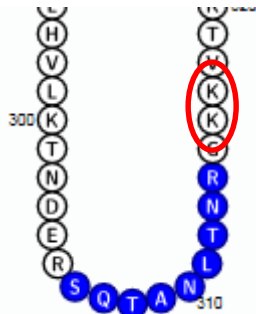


OATP1B3

■ Monkey Hepatocyte
■ Human Hepatocyte



Homological peptide in monkey and human



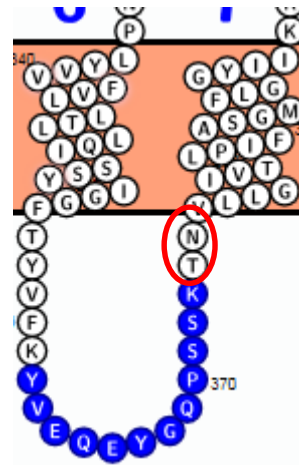
Continuous R/K
near cleavage site

monkey

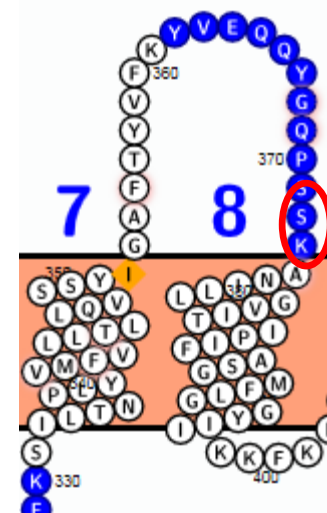
OATP1B3

Human

Distance to TMD longer



monkey



OATP1B1

Human