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APPENDIX

2017

Worked examples explain how to duplicate the figures and tables in the Report, using published data. Copies of the files described herein are available online (playingcomplexitygames.com).

APPENDIX I

HOMOGENEITY POSTULATES

Overview: The appendices make two assumptions. The first assumes that deDuve's postulates of biochemical homogeneity and single locations are correct and the second that Jacob Monot's famous quote "What's true for e coli is true for elephants," also means that "What's true for rat livers is true for livers in general." To test these assumptions, the appendices include both the data and calculations used to create the tables and figures included in the report. This gives the interested reader a working knowledge of what we can do with published data when we imagine biology to be a mathematical puzzle. Basically, it's becomes a lesson in how to play a smarter game.

Sometimes it's helpful to put things into perspective before plunging into the details. Consider this. Our research community is playing a zero-sum game (win-lose) with reductionism, which, according to statisticians, we are losing big time in that they have shown our results to be correct only 20% to 30% of the time. Since this is most likely the case, it appears that we are not playing our best game.

Several years ago, the EBSP (Enterprise Biology Software Project) found a similar shortfall and switched to a non-zero-sum game wherein two possible outcomes exist (win-win; lose-lose). The ensuing reports found repeatedly that by using parallel complexities, results inevitably ended up in the win-win column. The explanation offered for this unexpected largesse was that biology was behaving more like a mathematical puzzle than a mysterious complexity. Since the biology literature has focused largely on reducing biology to many little pieces, finding solutions became an exercise in reconstituting biology by putting some of the pieces back together. This process consisted of finding patterns, condensing them into equations, and scoring wins. The most difficult part of the job – running the experiments – had already been done and published, often many years ago. Recalculating results is surprisingly easy and can be quite rewarding.

The Appendix has two parts. The first focuses on the biochemistry postulates, the second on the postulate of biological homogeneity.

Files Used in the Appendix

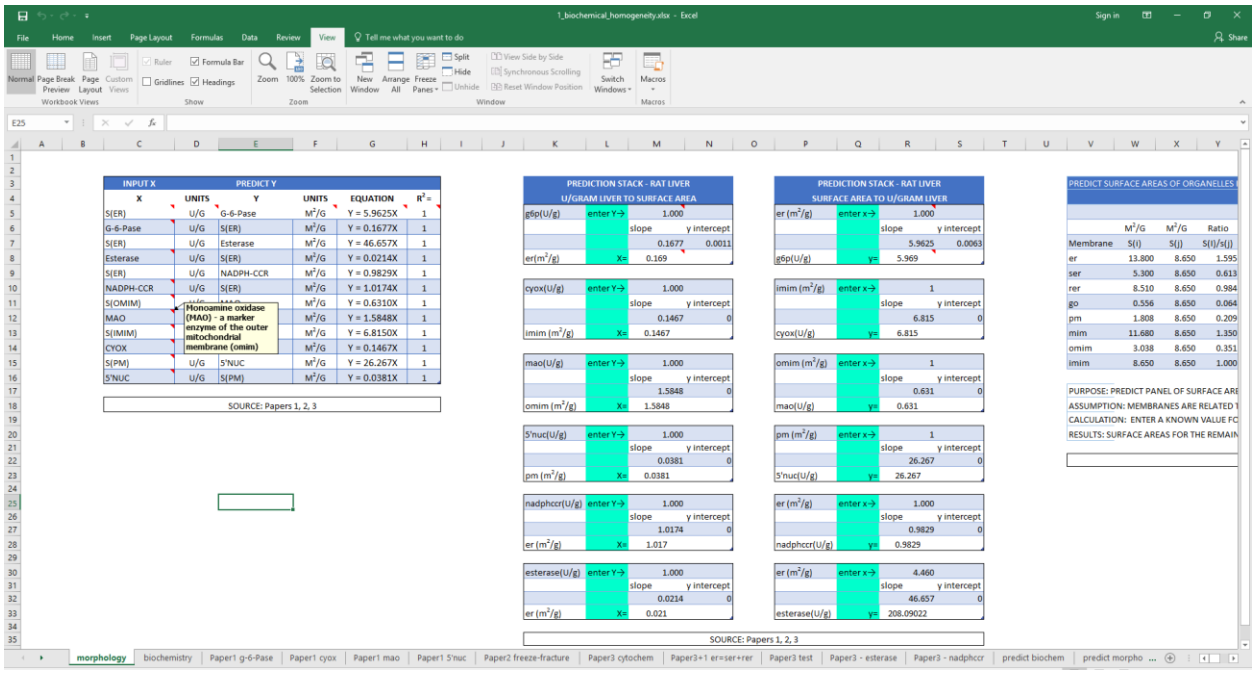
File	Program	Source
1_biochemical_homogeneity.xlsx	Excel	Microsoft
2_biological_homogeneity.xlsx	Excel	Microsoft
3_enzyme_triplets_amar(1974)_2017.xlsx	Excel	Microsoft
4_enzyme_triplets_amar(1974)_2017.nb	Mathematica 11	Wolfram
5_g6pase_triplets_amar(1974)_2017.nb	Mathematica 11	Wolfram

Postulates of Biochemical Homogeneity and Single Location

File: 1_biochemical_homogeneity.xlsx

Overview: Open the first Excel file (Microsoft) (1_biochemical_homogeneity.xlsx). The file includes 14 tabbed worksheets listed by paper (1-3) and by ascending figure and table number. Since most of the worksheets are interactive and self-explanatory (annotated), only selected images will be included here.

1. Morphology



INPUT X		PREDICT Y			
X	UNITS	Y	UNITS	EQUATION	R^2 =
S(ER)	U/G	G-6-Pase	M^2/G	Y = 5.9625X	1
G-6-Pase	U/G	S(ER)	M^2/G	Y = 0.1677X	1
S(ER)	U/G	Esterase	M^2/G	Y = 46.657X	1
Esterase	U/G	S(ER)	M^2/G	Y = 0.0214X	1
S(ER)	U/G	NADPH-CCR	M^2/G	Y = 0.9829X	1
NADPH-CCR	U/G	S(ER)	M^2/G	Y = 1.0174X	1
S(OMIM)	U/G	MAO	M^2/G	Y = 0.6310X	1
MAO	U/G	S(IMIM)	M^2/G	Y = 1.5848X	1
S(IMIM)	U/G	CYOX	M^2/G	Y = 6.8150X	1
CYOX	U/G	S(PM)	M^2/G	Y = 0.1467X	1
S(PM)	U/G	5'NUC	M^2/G	Y = 26.267X	1
5'NUC	U/G	S(PM)	M^2/G	Y = 0.0381X	1

Monoamine oxidase (MAO) - a marker enzyme of the outer mitochondrial membrane (omim)

SOURCE: Papers 1, 2, 3

Notice that some of the data entry fields carry a black triangle in the upper right hand corner. It signals the presence of a comment, which can be read by moving the cursor over the field – as shown at the left.

PREDICTION STACK - RAT LIVER U/GRAM LIVER TO SURFACE AREA			
g6p(U/g)	enter Y→	29.031	
		slope	y intercept
		0.1677	
er(m^2/g)	X=	4.870	

PREDICTION STACK - RAT LIVER SURFACE AREA TO U/GRAM LIVER			
er (m^2/g)	enter x→	4.870	
		slope	y intercept
		5.9625	0.0063
U/g)	y=	29.041	

The equation translates the marker enzyme activity into its equivalent amount of er surface area.

cyox(U/g)	enter Y→	1.000	
		slope	y intercept
		0.1467	0
imim (m^2/g)	X=	0.1467	

n (m^2/g)	enter x→	1	
		slope	y intercept
		6.815	0
cyox(U/g)	y=	6.815	

By expressing the relationship of morphology to biochemistry as an equation that passes through the origin, we can predict one from the other. Entering an enzyme activity for glucose-6-phosphatase (g9p) [29.031] into the enter Y→ field generates a specific amount of er membrane surface area [X = 4.870] (left side). Conversely, if you have a membrane surface area [e.g., enter x→ 4.870], you can predict how much g6p activity it produces [Y = 6.815].

PREDICT SURFACE AREAS OF ORGANELLES IN THE HEPATOCYTE (RAT LIVER) - CORRECTED FOR SECTION RELATED BIASES - CORRECT NEW DATA BEFORE ENTERING

Generate Ratios					Enter x ↓		Enter x ↓		Enter x ↓		Enter x ↓		Enter x ↓		Enter x ↓		Enter x ↓		Enter x ↓
	M^2/G	M^2/G	Ratio	er	4.6	ser	1	rer	1	go	1	pm	1	mim	1	omim	1	imim	1
Membrane	S(i)	S(j)	S(I)/s(j)																
er	13.800	8.650	1.595	1.000	4.600	2.604	2.604	1.622	1.622	24.820	24.820	7.633	7.633	1.182	1.182	4.542	4.542	1.595	1.595
ser	5.300	8.650	0.613	0.384	1.767	1.000	1.000	0.623	0.623	9.532	9.532	2.931	2.931	0.454	0.454	1.745	1.745	0.613	0.613
rer	8.510	8.650	0.984	0.617	2.837	1.606	1.606	1.000	1.000	15.306	15.306	4.707	4.707	0.729	0.729	2.801	2.801	0.984	0.984
go	0.556	8.650	0.064	0.040	0.185	0.105	0.105	0.065	0.065	1.000	1.000	0.308	0.308	0.048	0.048	0.183	0.183	0.064	0.064
pm	1.808	8.650	0.209	0.131	0.603	0.341	0.341	0.212	0.212	3.252	3.252	1.000	1.000	0.155	0.155	0.595	0.595	0.209	0.209
mim	11.680	8.650	1.350	0.846	3.893	2.204	2.204	1.373	1.373	21.007	21.007	6.460	6.460	1.000	1.000	3.845	3.845	1.350	1.350
omim	3.038	8.650	0.351	0.220	1.013	0.573	0.573	0.357	0.357	5.464	5.464	1.680	1.680	0.260	0.260	1.000	1.000	0.351	0.351
imim	8.650	8.650	1.000	0.627	2.883	1.632	1.632	1.016	1.016	15.558	15.558	4.784	4.784	0.741	0.741	2.847	2.847	1.000	1.000

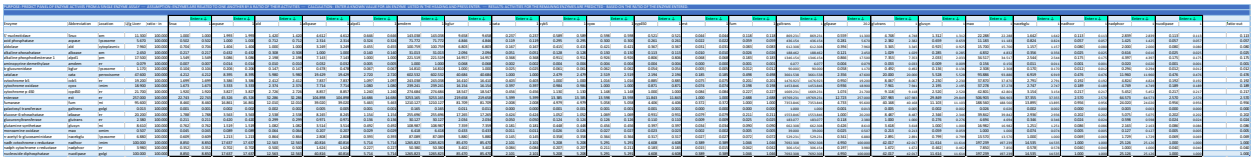
In a control setting, organelles occur in ratios characteristic of a given cell type. If, for example, a hepatocyte has 4.6 M^2/G, entering that value into the space provided predicts values for the remaining membrane organelles listed.

RAT LIVER - PREDICT RATIOS OF ORGANELLES - ONE CELL AT A TIME

	m^2/cm^3 paren	To calculate ratio enter value ↓	%	m^2/cm^3 paren	To calculate ratio enter value ↓	%	m^2/cm^3 paren	To calculate ratio enter value ↓	%	m^2/cm^3 paren	To calculate ratio enter value ↓	%
compartment i	**hepatocyte**	3.801	check	**endothelial cell**	1.000	check	**kupffer cell**	1.000	check	**fat-storing cell**	1.000	check
pm	0.563	0.148	3.5%	0.116	0.116	22.0%	0.033	0.033	11.9%	0.054	0.054	28.9%
er	3.801	1.000	23.7%	0.134	0.134	25.5%	0.080	0.080	29.1%	0.045	0.045	23.9%
rer	2.412	0.635	15.0%	0.081	0.081	15.4%	0.061	0.061	22.1%	0.036	0.036	19.1%
ser	1.389	0.365	8.6%	0.053	0.053	10.1%	0.019	0.019	7.0%	0.009	0.009	4.9%
mi	3.825	1.006	23.8%	0.049	0.049	9.3%	0.027	0.027	9.9%	0.020	0.020	10.8%
omim	1.300	0.342	8.1%	0.020	0.020	3.8%	0.009	0.009	3.2%	0.007	0.007	3.9%
imim	2.525	0.664	15.7%	0.029	0.029	5.5%	0.018	0.018	6.7%	0.013	0.013	6.7%
go	0.170	0.045	1.1%	0.024	0.024	4.6%	0.004	0.004	1.5%	0.003	0.003	1.3%
ly	0.070	0.018	0.4%	0.012	0.012	2.3%	0.020	0.020	7.3%	0.001	0.001	0.5%
pinoves	0.011	0.003	0.1%	0.008	0.008	1.6%	0.003	0.003	1.2%	0.000	0.000	0.1%
∑	16.066	4.227	100.0%	0.526	0.526	100.0%	0.274	0.274	100.0%	0.187	0.187	100.0%

The liver contains four parenchymal cell types, wherein each cell displays organelles in proportions specific to its cell phenotype. In turn, intracellular and intercellular proportions of organelles – along with the cell frequencies - define the phenotype of the liver parenchyma. This means that we can generate a control liver parenchyma quantitatively from a single estimate for a membrane surface area or a marker enzyme activity.

2. Biochemistry



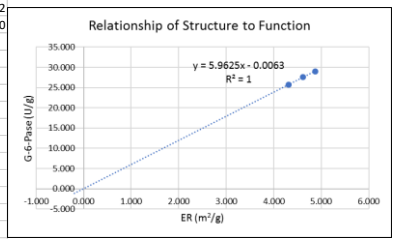
PURPOSE: PREDICT PANEL OF ENZYME ACTIVIES FROM A SINGLE ENZYME ASSAY --- ASSUMPTION: ENZYMES ARE RELATED TO ONE ANOTHER BY A RATIO OF THEIR ACTIVITIES -

						Enter x ↓		Enter x ↓		Enter x ↓	
Enzyme	Abbreviation	Location	U/g Liver	ratio - in	5nuc	1	acpase	1	ald	1
5'-nucleotidase	5nuc	pm	11.300	100.000	1.000	1.000	1.993	1.993	1.420	1.420
acid phosphatase	acpase	lysosome	5.670	100.000	0.502	0.502	1.000	1.000	0.712	0.712
aldolase	ald	cytoplasmic	7.960	100.000	0.704	0.704	1.404	1.404	1.000	1.000
alkaline phosphatase	alkpase	pm	2.450	100.000	0.217	0.217	0.432	0.432	0.308	0.308
alkaline phosphodiesterase 1	alpd1	pm	17.500	100.000	1.549	1.549	3.086	3.086	2.198	2.198
aminopyrine demethylase	amdem	er	0.079	100.000	0.007	0.007	0.014	0.014	0.010	0.010
b-glucuronidase	bglur	lysosome	1.170	100.000	0.104	0.104	0.206	0.206	0.147	0.147
catalase	cata	peroxisome	47.600	100.000	4.212	4.212	8.395	8.395	5.980	5.980
cytochrome b5	cyb5	er	19.200	100.000	1.699	1.699	3.386	3.386	2.412	2.412
cytochrome oxidase	cyox	imim	18.900	100.000	1.673	1.673	3.333	3.333	2.374	2.374
cytochrome p 450	cyp450	er	21.700	100.000	1.920	1.920	3.827	3.827	2.726	2.726
esterase	est	er	257.000	100.000	22.743	22.743	45.326	45.326	32.286	32.286
fumarase	fum	mi	95.600	100.000	8.460	8.460	16.861	16.861	12.010	12.010
galactosyl transferase	galtrans	golgi	0.013	100.000	0.001	0.001	0.002	0.002	0.002	0.002
glucose-6-phosphatase	g6pase	er	20.200	100.000	1.788	1.788	3.563	3.563	2.538	2.538
glucuronyltransferase	glutrans	er	2.380	100.000	0.211	0.211	0.420	0.420	0.299	0.299
glutamine synthetase	glusyn	mi	8.610	100.000	0.762	0.762	1.519	1.519	1.082	1.082
monoamine oxidase	mao	omim	0.507	100.000	0.045	0.045	0.089	0.089	0.064	0.064
n-acetyl-b-glucosaminidase	nacebglu	lysosome	6.880	100.000	0.609	0.609	1.213	1.213	0.864	0.864
nadh cytochrome c reductase	nadhccr	imim	100.000	100.000	8.850	8.850	17.637	17.637	12.563	12.563
nadph cytochrome c reductase	nadphccr	er	3.980	100.000	0.352	0.352	0.702	0.702	0.500	0.500
nucleoside diphosphatase	nucdipase	golgi	100.000	100.000	8.850	8.850	17.637	17.637	12.563	12.563

This table uses the published data Amar-Costesec et al., (1974) to generate ratios that predict enzyme activities for 21 marker enzymes from a single control value.

3. Paper1g-6-Pase

Paper 1	g-6-pase	H=	27.421	U/g									
OBJECTIVE: To integrate stereological and biochemical data mathematically with the goal of developing and testing prediction models.													
DATA SOURCE: Bolender et al., 1978. J Cell Biol. 77:565-583.													
STEP 1: Define the relationship of structure to function with an equation with an R^2=1.								STRATEGY: Distribute averaged biochemical data (n=3) to indivi					
T	tissue			membrane	enzyme								
H	homogenate			surface	activity								
F	fractions			m2/g liver	U/g liver								
				T	H		H	H	H		T	H	
				er	g-6-pase		g-6-pase	g-6-pase	g-6-pase		m2/g liver	g-6-pase	
		animal 1	er-1	4.870	9.677		0.353	82.263	29.031		4.870	29.031	
		animal 2	er-2	4.310	8.564		0.312	82.263	25.692		4.310	25.692	
		animal 3	er-3	4.620	9.180		0.335	82.263	27.540		4.620	27.540	
				13.800	27.421		1.000		82.263				
				4.6					27.421				
		4.87x+4.31x+4.62x=27.422											
		13.8x=27.422											
		x=		1.9871									
		er	g-6-pase										
		4.87*x=	9.677										
		4.31*x=	8.564										
		4.62*x=	9.180										
			27.422										



The next series of tabs – named after marker enzymes – explain how to translate published biological data into R^2 = 1 equations. Such equations support the biochemical postulates and quantify prediction. Comments – under the black triangles – annotate the calculations.

7. Paper2 freeze-fracture

	P		
er	2.473	2.811464	88.0%
omim	0.132	2.811464	4.7%
pm	0.139	2.811464	5.0%
imim	0.067	2.811464	2.4%
	2.811	2.811464	100.0%
PM+IMIM	0.207	2.811464	7.4%
ER	2.473	2.811464	88.0%
OMIM	0.132	2.811464	4.7%
	2.811		100.0%

	PM+IMIM	ER	OMIM
Freeze-Fracture	20.1%	63.0%	17.0%
Predicted from Enzy	7.4%	88.0%	4.7%

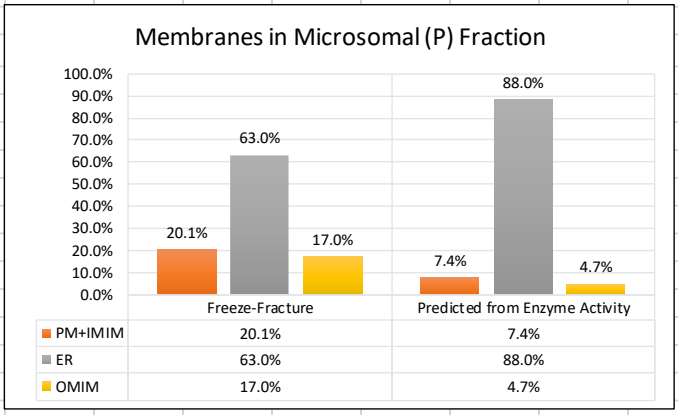
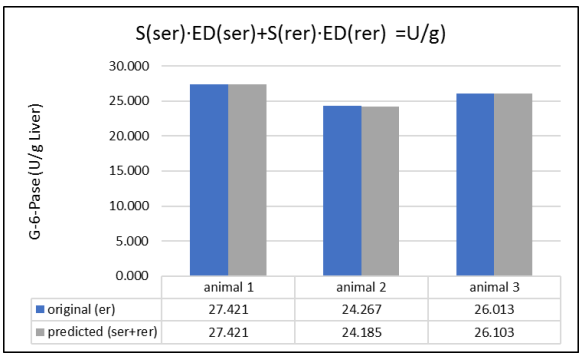
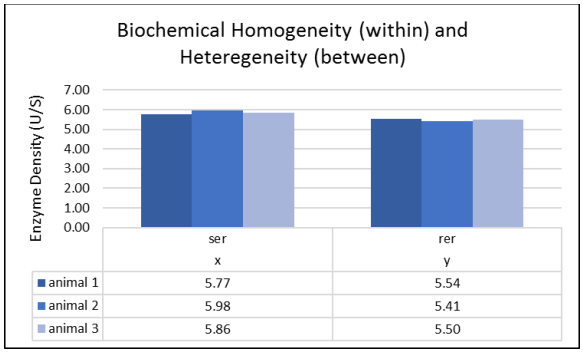


Figure 10

The original experiment of Losa et al., (1978) was rerun, this time predicting the membrane surface areas of organelles from their marker enzyme activities, as measured in the P (microsomal) fraction.

9. Papers3+1 er=ser+rer

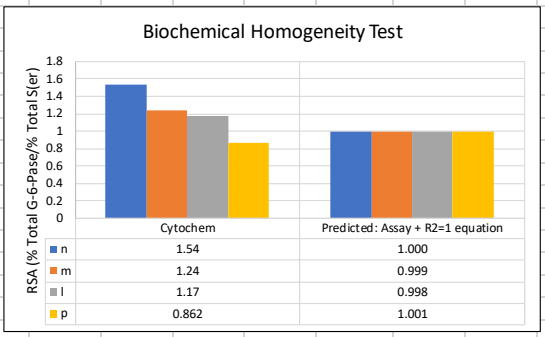




Pairs of linear equations in x and y can be solved simultaneously to detect the concentration of a given marker enzyme at two different morphological locations. Such equations also serve as a convenient test for the homogeneity postulate. The equations were evaluated in Mathematica.

10. Paper3 test

	G-6-Pase										
	Cytochem	Predicted: Assay + R^2=1 equation									
n	1.54	1.000									
m	1.24	0.999									
l	1.17	0.998									
p	0.862	1.001									



			g6pase	er		S(er)		g6pase	S(er)	
	G-6-Pase		% of Total	Predicted		% of Total		% of Total	% of Total	RSA
n	4.230	22.680	18.65%	0.710	3.807836	18.66%		18.65%	18.66%	1.000
m	3.000	22.680	13.23%	0.504	3.807836	13.24%		13.23%	13.24%	0.999
l	2.150	22.680	9.48%	0.362	3.807836	9.50%		9.48%	9.50%	0.998
p	13.300	22.680	58.64%	2.232	3.807836	58.60%		58.64%	58.61%	1.001
	22.680	22.680	100.00%	3.808	3.807836	100.00%		100.00%	100.00%	1.000

g6p(U/g)	enter Y→	13.300	
		slope	y intercept
		0.1677	0.0011
er(m^2/g)	X=	2.232	

The original study detected a heterogeneous distribution of glucose-6-phosphatase on the membranes of the ER in the four fractions (N, M, L, P), whereas the updated one found homogeneity.

File: 2_biological_homogeneity.xlsx

Overview: Open the second Excel file (Microsoft) (2_biological_homogeneity.xlsx). The file includes 4 tabbed worksheets listed by paper. It includes the data and calculations used to livers from three species – human, dog, and rat.

1. Koch-1978-human vs rat
2. Roessner-1978-human vs rat
3. de-la-Iglesia-1976-human vs rat
4. Hess-1973-dog vs rat

File: 2_biological_homogeneity.xlsx

Overview: Open the second Excel file (Microsoft) (2_biological_homogeneity.xlsx). The file includes 4 tabbed worksheets listed by paper. It includes the data and calculations used to livers from three species – human, dog, and rat.

File: 3_enzyme_triplets_amar(1974)_2017.xlsx

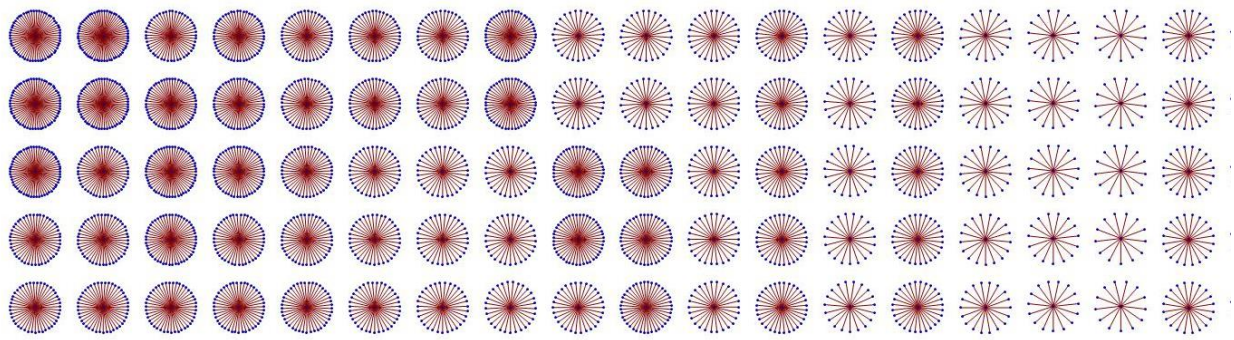
Overview: Open the third Excel file (Microsoft) (3_enzyme_triplets_amar(1974)_2017.xlsx. It includes the data of Amar-Costesec et al. (1974) fitted to triplets (mathematical markers and connection ratios). Here the goal was to identify quantitative patterns being shared by different marker enzymes.

"	5nucl1acidpase0.5aldoase0.7	"->"	part1part0.5part0.7	",
"	5nucl1acidpase0.5alkapase0.2	"->"	part1part0.5part0.2	",
"	5nucl1acidpase0.5alkapdiestase11.5	"->"	part1part0.5part1.5	",
"	5nucl1acidpase0.5aminopdemease0.007	"->"	part1part0.5part0.007	",
"	5nucl1acidpase0.5bglucase0.1	"->"	part1part0.5part0.1	",
"	5nucl1acidpase0.5catalase4	"->"	part1part0.5part4	",
"	5nucl1acidpase0.5chol0.2	"->"	part1part0.5part0.2	",
"	5nucl1acidpase0.5cytob51.5	"->"	part1part0.5part1.5	",
"	5nucl1acidpase0.5cytoox1.5	"->"	part1part0.5part1.5	",
"	5nucl1acidpase0.5cytop4501.5	"->"	part1part0.5part1.5	",
"	5nucl1acidpase0.5esterase20	"->"	part1part0.5part20	",
"	5nucl1acidpase0.5fumaase8	"->"	part1part0.5part8	",
"	5nucl1acidpase0.5g6pase0.001	"->"	part1part0.5part0.001	",
"	5nucl1acidpase0.5galatransase1.5	"->"	part1part0.5part1.5	",
"	5nucl1acidpase0.5gluctransase0.2	"->"	part1part0.5part0.2	",
"	5nucl1acidpase0.5glutsynase0.7	"->"	part1part0.5part0.7	",
"	5nucl1acidpase0.5mao0.04	"->"	part1part0.5part0.04	",
"	5nucl1acidpase0.5nacetylglucase0.6	"->"	part1part0.5part0.6	",
"	5nucl1acidpase0.5nadhccr8	"->"	part1part0.5part8	",
"	5nucl1acidpase0.5nadphcccr_er0.3	"->"	part1part0.5part0.3	",

Special formatting was added to the data set so that it could be plotted in Mathematica.

File: 4_enzyme_triplets_amar(1974)_2017.nb File: 5_g6pase_triplets_amar(1974)_2017.nb

Overview: Mathematica plots of data stored in File 3. Note: Software can be downloaded from Mathematica to view these plots.



The plots display massive connectivity among liver enzymes.