Metabolome Analysis and Systems Biology

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Keio University Facts (K.O.)

Founded in 1858, Keio is the oldest institution of higher education in Japan...

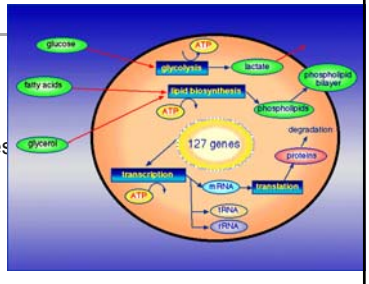
Undergraduate Students: 28,124
Graduate Students (Masters): 2,367
Graduate Students (Doctorate): 948

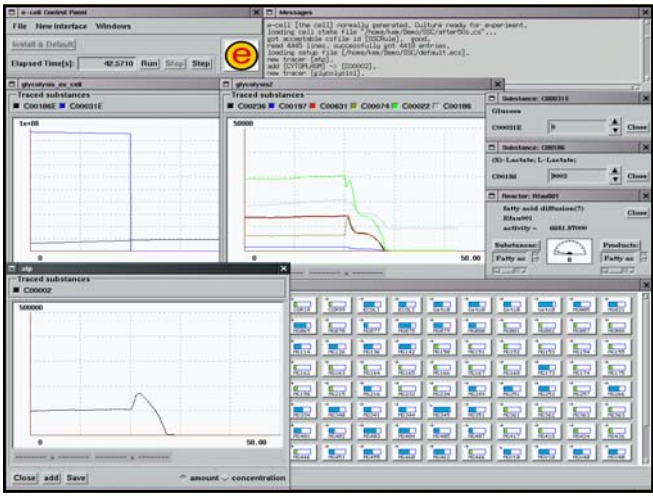
-5 campuses
-8 faculties
(22 departments)
-9 graduate schools

The first "e-Cell"

(Tomita *et al.* 1997)

- It has:
  - **127** genes
  - **4268** molecular species
  - **495** reactions
- It performes:
  - glycolysis
  - lipid synthesis
  - transcription, translation and degradation





BIOINFORMATICS

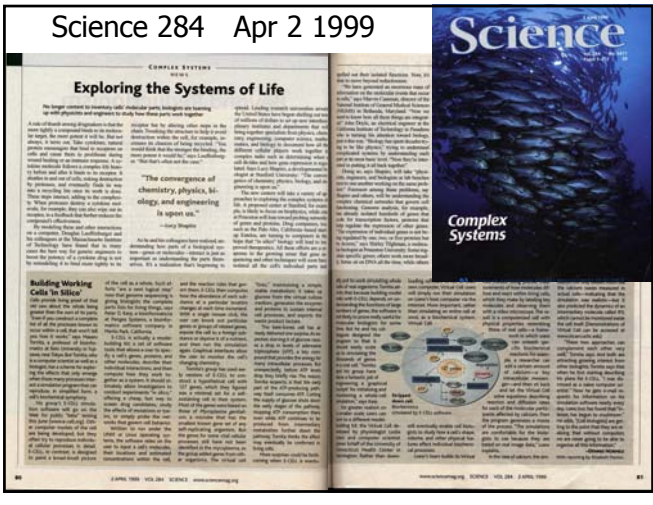
Bioinformatics, Vol 15, 72-84, Copyright © 1999 by Oxford University Press

ARTICLES

E-CELL: software environment for whole-cell simulation

M Tomita, K Hashimoto, K Takahashi, TS Shimizu, Y Matsuzaki, F Miyoshi, K Saito, S Tanida, K Yugi, JC Venter and CA Hutchison 3rd

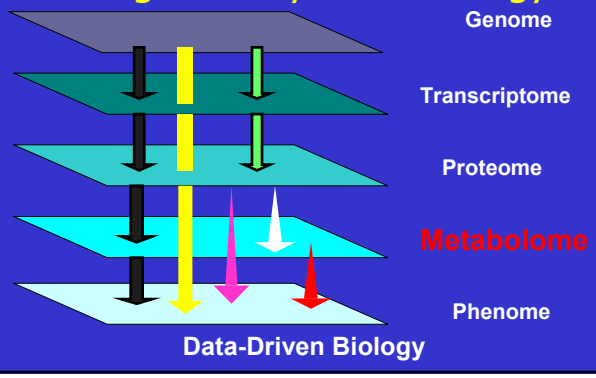
Science 284 Apr 2 1999



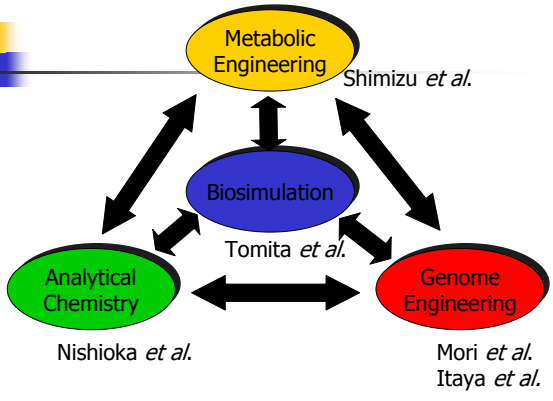
Lack of quantitative data: Needs for Metabolome Project

- Systematic and comprehensive analysis of metabolic data
  - Steady state concentration
  - Kinetic parameters
  - Flux rates etc.
- High-throughput measurement devices

Integrative Systems Biology



Institute for Advanced Biosciences Keio University



Quantitative Metabolomics CE/MS: Soga *et al.*

More than1000 peaks
in 30 minutes, 45 samples a day
Sample amount: 10 nl, Error rate: +-8%

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Research Articles

May 15, 2002 / Volume 74 / Issue 10

Current Issue:
Volume 74, Issue 10 (May 15, 2002)
Editor Royce W. Murray & Associate Editors

ACCELERATED ARTICLES

2233-2239 **Simultaneous Determination of Anionic Intermediates for *Bacillus subtilis* Metabolic Pathways by Capillary Electrophoresis Electrospray Ionization Mass Spectrometry** *Tomoyoshi Soga, Yuki Ueno, Hisako Naraoka, Yoshiaki Ohashi, Masaru Tomita, and Takaaki Nishioka* [Abstract][Full Text - HTML][Full Text - PDF][Purchase Article]

2240-2247 **Particles for Multiplexed Analysis in Solution: Detection and Identification of Striped Metallic Particles Using Optical Microscopy** *Ian D. Walton, Scott M. Norton, Arjuna Balasingham, Lin He, Dominador F. Oviso, Jr., Dimpy Gupta, Paul A. Raju, Michael J. Natan, and R. Griffith Freeman* [Abstract][Full Text - HTML][Full Text - PDF][Purchase Article]

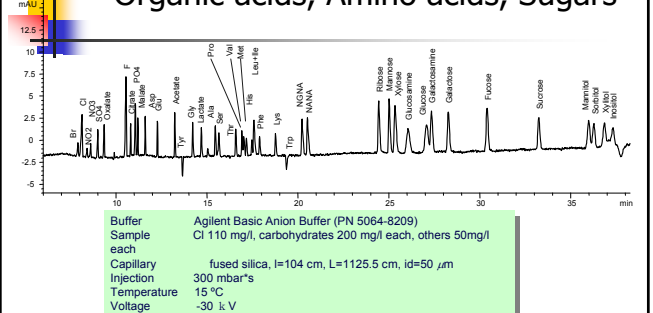
ARTICLES

2248-2256 **Triplet State Photoexcitation Dipole Jump Relaxation Method To Observe Adsorption/Desorption Kinetics at a Reversed-Phase Silica/Solution Interface** *Stephanie R. Shield and Joel M. Harris* [Abstract][Full Text - HTML][Full Text - PDF][Purchase Article]

2257-2260 **Ultratrace Analysis Based on Hadamard Transform Capillary Electrophoresis** *Takashi Kaneta, Kazuki Kosai, and Totaro Imasaka*

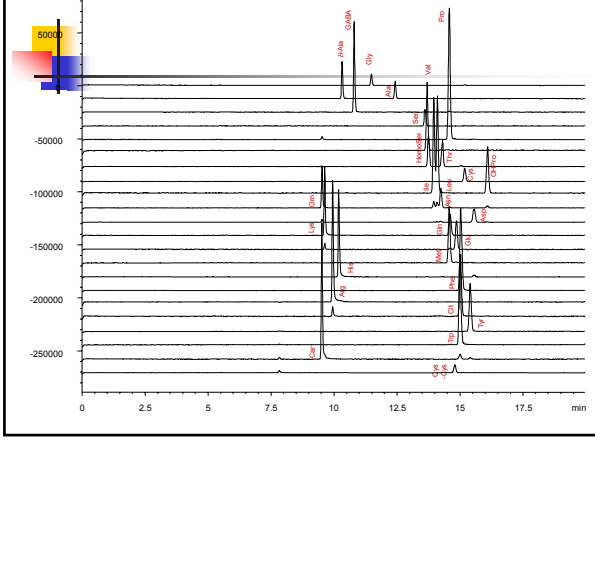
analytical chemistry

Capillary Electrophoresis: Organic acids, Amino acids, Sugars

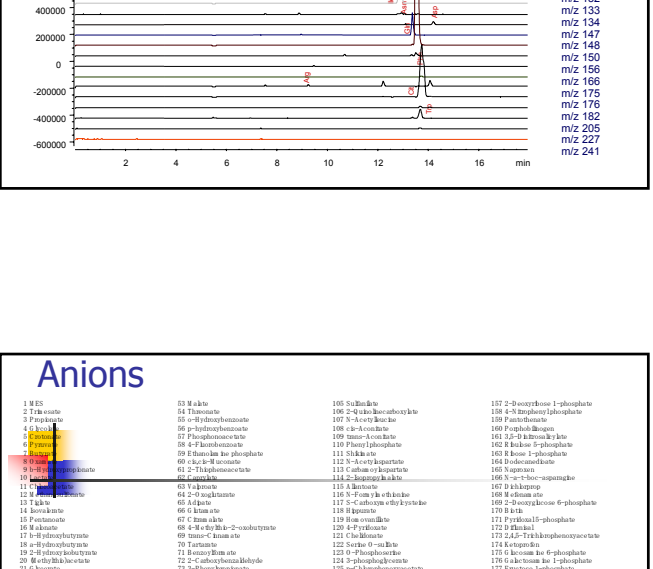


Buffer	Agilent Basic Anion Buffer (PN 5064-8209)
Sample	Cl 110 mg/l, carbohydrates 200 mg/l each, others 50mg/l each
Capillary	fused silica, l=104 cm, L=1125.5 cm, id=50 µm
Injection	300 mbar*s
Temperature	15 °C
Voltage	-30 k V
Detection	Sig. 350/20 nm, Ref. 230/10 nm

CE/MS: amino acids (100µM)



CE/MS: amino acids in *B.subtilis*



Cations

1 3-Aminopropiohydrile sodium nitrate salt	74 Nicotinate ion	147 4-Aminobenzoic acid	220 Phosphorylcholine chloride	293 5-Methylcytosine
2 2-Aminoethanesulfonic dihydrochloride	75 Nicotinamide	148 Hydroxyproline	221 Dopamine HCl	294 Kynurenine
3 Betaine	76 2-Chloro-2-adenine hydrochloride	149 Propionic acid hydrochloride	222 Deoxycytidine	295 Tobramycin
4 Acetyl-beta-methylcholine	77 Pyrimidine	150 Sec-Butylamine hcl	223 Anthrone	296 L-o-O-(o-phosphorylcholine) 1,5 codeine chloride
5 Aminoguanidine hcl	78 5-Methylcytosine	151 Pseudoephedrine	224 N,N-diethylaniline diethylenediamine	297 Ethanolamine
6 Tetramethylammonium chloride	79 1-Methylhistamine dihydrochloride	152 L-Histidine	225 Sec-Butylcyclohexylamine	298 NG-Methyl-5-C-deoxyadenosine
7 Cytosine ion hydrochloride	80 Imidazole-4-acetate sodium salt	153 L-Ile-D-Ala dihydrochloride hcl	226 Cytosine	299 4,4'-Methylene bis(N-ethylaniline)
8 Tetraethylammonium chloride	81 Histamine	154 2-Aminoethanethiol	227 L-Alanine methylester hydrochloride	300 Adenosine
9 Choline chloride	82 Ethylenediamine dihydrochloride BHD	155 Ethylenediamine	228 Cytosine arabinoside	301 Adenosine monophosphate
10 Tetraethylammonium bromide	83 L-Ornithine hcl	156 Acetamidine hcl	229 Carbamoylcholine	302 H,-Gly-Gly-Gly
11 Choline bromide	84 Imipenem hcl	157 Adenosine	230 DL-a, a'-Diaminopimelic DL-2,6-Diaminohepta	303 Inosine
12 Tetraethylammonium iodide	85 Spermidine	158 Indole-3-acetaldehyde-sodium bisulfite addition com	231 5-Methoxytryptamine hcl	304 Kanamycin (phosphate free)
13 Tigloidine	86 1-Aminoethylphosphonic acid (Cyclidine)	159 N-Methyl-N-propargylbenzylamine	232 Carbamylcholine	305 G-Ala
14 Tetraethylammonium iodide	87 Tetramethylammonium chloride	160 Methyl-beta-methylcholine	233 Cytosine-beta-D-arabinofuranoside	306 Kanamycin sulfate
15 L-Ornithine	88 Hydroxylamine hcl, 2-Methylpiperazine hydrochloride	161 Bromocholine	234 Caffeine	307 Gentamicin
16 L-Histidine	89 O-Methylcholine hydrochloride	162 Tetrabutylammonium(n)2-Aminoethyl hydrochloride	235 D-Glucosamine hcl, acid	308 Lactobacillus
17 β-Alanine	90 N-Acetylpiperazine hydrochloride	163 Tryptamine	236 L-Tyrosine methyl ester	309 Enoxacin 1,8-Diethyl-1,4,5,8-tetrahydroquinoline,[3,4-]
18 Sarcosine	91 Agmatine	164 O-Acetylcholine hydrochloride	237 L-β-(3,4-dihydroxyphenyl)alanine DOPA	310 Atropine
19 Acetyl choline hydrochloride	92 L-Hydroxylysine	165 L-o-Aminoethyl acid	238 N-Acetyl-L-thrifine	311 L-Histidinol dihydrochloride
20 2-Hydroxypyridine	93 Creatinine monohydrate	166 N-Methyl-L-glutamic acid	239 Hydroxytryptamine	312 Tobramycin
21 Tetramethylammonium chloride	94 Tetraethylammonium chloride	167 Tetraethylammonium tetrafluoroborate	240 Tetrabutylammonium tetrafluoroborate	313 Tobramycin
22 1-Methyl-2-pyrrolidinone	95 o-Acetylcarnitine (R-isomer)	168 L-Cysteine	241 Thiabendazole, standard	314 Kifalinone, sodium salt
23 Cytidine hcl hcl	96 L-Ornithine hcl	169 2-Dimethylaminoethyl hcl, dihydrochloride	242 Silicotine	315 7-Deoxy-C-Methylthiokanosine
24 o-Acetylcarnitine(1-carboxylic	97 L-Lysine hcl	170 4-Methylpyridine hydrochloride	243 L-Tryptophanamide hydrochloride	316 5-Acetylcholine hydrochloride
25 L-Acetylcarnitine(2-carboxylic acid)	98 L-Histidine hcl	171 Acetylcholine	244 O-Acetyl-L-carnitine hydrochloride	317 Vancomycin
26 Cytosine	99 Tetraethylammonium bromide	172 Cytosine	245 Tetramethylammonium chloride	318 Vancomycin
27 Cadaverine(1,5-Diaminopentane) dihydrochloride	100 L-Arginine monohydrate	173 Proline	246 5-Methoxy-3-dehydroacetic acid	319 N-a-Benzoyl-L-arginine ethylester
28 Biotine	101 G-Arginine	174 2-Methylpiperazine	247 L-Glutamine (Mixture of two enantiomers)	320 2-Indol-L-tryptophan
29 o-Acetylcarnitine (GABA)	102 o-Ala-Ala-Ala	175 2-Methylpiperazine	248 Hikizolid	321 Kifalinone, reduced form
30 o-Ala-Ala-Ala-Ala-Ala	103 D-Ala-Ala-Ala	176 Benzylamine	249 Hikizone	322 Bacitracin
31 N,N-Dimethylaniline	104 L-N-Formylkynurenine monohydrate	177 L-N-Phenylalanine	250 Zidkhidine	323 Tobramycin sulfate
32 N-Methylbenzylamine	105 L-Asparagine	178 Ethylamine hcl	251 Rac-Bilin	324 Chlamydocidin
33 Methylbenzylamine	106 Acetylcholine	179 Dimethylaminoethylene hydrochloride	252 Dimethylaminoethylene hydrochloride	325 Kifalinone
34 DL-2,3-Diaminopropionic	107 DL-Glutamic acid	180 Epidine hydrochloride	253 Rumicine	326 Kifalinone epoxide a-alone salt
35 L-Serine	108 Adenine	181 Phenylpiperidine hydrochloride	254 D-Desthiobiotin	327 Fluoxine
36 N-Methylaniline	109 Acetamidine	182 Synepidine	255 6-Furfuryladenine hemiprate	328 Spectinomycin dihydrochloride pentahydrate
37 Phenylpyridine	110 Hypoxanthine	183 Nicotinamide hydrochloride	256 β-Alanine	329 Rotherbine
38 Methylaniline	111 Dimethylamine hydrochloride	184 Tetramethylammonium hydrochloride	257 Cytosine-5,5-dihydrouracil	330 Kifalinone-diethylbrine
39 Methylaniline	112 Pyridinium chloride	185 L-Nicotinamide hydrochloride	258 Cytosine-5,5-dihydrouracil	331 2-Amino-4-(4-hydroxy-4-methylpiperidyl)-6-methoxy
40 2-Aminoethanol	113 Acetamidine	186 Pyridoxine	259 Tomo-Zoxin	332 Tilt (Tilt)
41 2-Aminoethanol	114 p-Ala-Ala-Ala-Ala	187 2-Methylbenzylamine	260 5-Methoxy-L-tryptophan	333 Fabrazine
42 Cytosine	115 Isoniazid acid hydrazide	188 Diphenylamine	261 2-Acetamido-1-O-ara-1,2-dideoxy-β-D-glucopyra	334 Pentopine hydrochloride
43 4-Fluoroaniline	116 DL-2-Hydroxypropionic ala ha	189 Benzylamine	262 N-Acetyl-D-glucosamine	335 L-Tetrahydropalmatine
44 Hitman ha, diethylaniline	117 Tyrosine	190 Dodecylpyridinium	263 L-N-Cyanoethylamine	336 G-44
45 Cytosine	118 Tetraethylammonium hydrochloride	191 Tetrabutylammonium 4,2,2,6-tetrahydropyrrolidine hydro	264 Tetramethylammonium	337 Tetrapentapine
46 Cytosine hydrochloride	119 Uracil-acetic (N-Methylthiouracyl)	192 Indole-3-acetamide	265 Acrylamide	338 N-Acetylglutathione
47 2-Benzoyl-1-a-ethylbenzoate	120 G-Hydroxyisobutyric	193 N-Acetyl-L-carnitine	266 6-Benzylpyridine	339 Thiamine monophosphate chloride
48 6-Cytosine-1-a-ethylbenzoate	121 Tryptamine	194 N-Ethyl-L-alanine	267 Cyanoethyl	340 5-Methoxy-N,N-dimethylbenzylamine
49 Nicotinic	122 Hexamethylene tetramine	195 L-N-Arginine	268 Hetrapyrone	341 5-Methoxy-N,N-dimethylbenzylamine
50 L-N-Proline	123 L-Histidine dihydrochloride	196 Gamma ha	269 Deoxycytidine	342 Leu-Leu-Ley
51 Guanidinoacetic acid	124 Tropine	197 N-Alanyl-a-ethylpyrazine ha	270 2'-Deoxycytidine	343 Bacitrifin
52 Guanidinoacetic acid	125 Tryptamine	198 Methylpyridinium iodide	271 Cytosine-5,5-dihydrouracil	344 Kifalinone-beta methyl-a-methylester
53 N-(2-Aminoethyl)-2-aminoethanol acid hydrate	126 4-Methylbenzylamine	199 Indole-3-acetic acid	272 Antimycine	345 12-N-Ethyl-1,5-tetrahydroxy dihydrate
54 Betaine,Adenine	127 Spermidine	200 2-Guanidinobenzimidazole	273 Herbicide	346 5-Iron ion sulfate salt
55 L-Serine	128 L-N-O-Acetyl-ha	201 L-C-Imidine	274 Lidocaine	347 Ke-thermosine
56 L-Valine	129 Ethylbenzylamine (Mixture)	202 L-Carnosine	275 Sarpagandol-B(R)	348 Riccarin p-b
57 Methylbenzylamine	130 Tetramethylammonium	203 Indole-3-acetic hydrochloride	276 Indole-3-acetic hydrochloride	349 Kanamycin
58 L-Q-Ala-Ala-Ala-Ala-Ala-Ala	131 Acetylcholine chloride	204 Allin	277 Fibin acid	350 Tobramycin
59 DL-6-Amino-3-hydroxybutyric acid	132 Triethylbenzylammonium ion	205 Cytosine	278 Ketamine	351 Potassium p-b
60 G-Aminoacetate	133 L-Ala-Ala-Ala	206 D-5-Aminoacetamide	279 N-Ethyl-2'-deoxycytidine	352 Kalamycin
61 o-Methylthiol-serine	134 Carbachol	207 G-Acetamide hydrochloride	280 L,D-Hydroxybutylcarbodiimide	353 Kanamycin monosulfate gamma-irradiated
62 L-Threonine	135 N-Methylnicotinamide	208 N-Aminoacetamide hydrochloride	281 Cytidine	354 Kanamycin sulfate
63 Purine	136 DL-Alanine-β-Hydroxyaspartic acid	209 Fumarate	282 Uridine	355 Bacitrifin
64 Tetramethylammonium hydroxide	137 Tetraethylammonium	210 Tetramethylammonium sulfate ha	283 Uridine	356 Kifalinone-b-sulfate
65 L-Cysteine	138 D-Proline Ala-Ala	211 Theophylline	284 Pyridoxine-5'-phosphate	357 Dihydroxypropanyl-ke
66 Benzamide	139 N-Methylcholine	212 o-Phenylcarbine	285 4-Ala-hydroxyfuroline	358 Kifalinone, oxidized form
67 2-Ala-Ala-2-Hydroxyethyl-Q-1,2-propanediol	140 O-Methylcholine hydrochloride	213 2-Ala-Ala-2-Hydroxy-phenylpropionic acid	286 Nicotinic acid	359 Kanamycin Sulfate
68 N,N-Dimethylaniline	141 L-Methylcholine	214 L-1-Tyrosine	287 2'-Deoxyadenosine monohydrate	360 Papain on gelatin sulfate
69 Methylbenzylamine	142 Tetraethylammonium	215 Tetrazole	288 Deoxyadenosine	361 5,2'-7-Diindole-5-thymosine
70 2,4-Ala-Ala-Ala-Ala	143 L-Ala-Ala-Ala-Ala	216 o-o-Ala-Ala-Ala-Ala	289 Purine Adenine	362 O-Sorbitol p-b
71 DL-5-a-Methylbenzylamine ha 5-(1-phenylethyl)	144 Guanine	217 Dacarbazine	290 Valpar	
72 p-Phenylethylamine Phenethylamine	145 2-Aminoacetamide ha hydrochloride	218 N,N-Diphenylethylene 4-(2-Hydroxyl-2-Ala	291 Pencilbrin	
73 Isoniazid ha	146 G-Aminoproprionic acid ethyl ester	219 N,N-Methylethylbrine-ethyl	292 Phosphoo-L-arginine	

Anions

1 MES	53 Malate	105 Sulfinilate	157 2-Deoxyribose 1-phosphate
2 Tris acetate	54 Thiosorate	106 2-Quinolinecarboxylate	158 4-Nitrophenyl phosphate
3 Propionate	55 o-Hydroxybenzoate	107 N-Acetylleucine	159 Pantethionate
4 Glycolate	56 p-hydroxybenzoate	108 cis-Aconitate	160 Porphobilinogen
5 Crotonate	57 Phosphoenolacetate	109 trans-Aconitate	161 3,2-Dihydroxybiphenyl
6 Pyruvate	58 4-Fluorobenzoate	110 Phenylphosphate	162 R-Ribose 5-phosphate
7 Butyrate	59 Ethanolamine phosphate	111 Shikimate	163 R-Ribose 1-phosphate
8 Oxalate	60 cis,z-8-Enoate	112 N-Acetylaspartate	164 Dodecanedioate
9 b-Hydroxybutyrate	61 2-Thiophenecarboxylate	113 Carbamoylaspartate	165 Naproxen
10 Lactate	62 Cinnamate	114 2-Isopropylmalate	166 N-a-t-boc-asparagine
11 Chloroacetate	63 Valpamate	115 Allantoate	167 D-Ribotropo
12 Malonate	64 2-O-tosylmaate	116 N-Formylethionine	168 Rifamycin
13 Tiglate	65 Adipate	117 S-Carboxymethylcysteine	169 2-Deoxyglucose 6-phosphate
14 Isovalerate	66 Glutamate	118 Hippurate	170 Biotin
15 Pentanoate	67 Cinnamate	119 Itaconomalate	171 Pyridoxal5-phosphate
16 Malonate	68 4-Methylthio-2-oxobutyrate	120 4-Pyridoxate	172 Biotinal
17 b-Hydroxybutyrate	69 trans-Cinnamate	121 Cholinate	173 2,4,5-Trichlorophenoxyacetate
18 a-Hydroxybutyrate	70 Tartarate	122 Serine O-sulfate	174 Ketoprofen
19 2-Hydroxyisobutyrate	71 Benzoylform ate	123 O-Phosphoserine	175 G-Ketosamine 6-phosphate
20 Methylthioacetate	72 2-Carboxybenzaldehyde	124 2-Phosphoglycerate	176 G-Galactosamine 1-phosphate
21 Glycerate	73 3-Phenylpropionate	125 p-Chlorophenoxyacetate	177 Fructose 1-phosphate
22 Pyruvate-2-carboxylate	74 X-Methylnorthiamine	126 Undecanoate	178 Fructose 6-phosphate
23 Methylsulfate	75 Phenoxyacetate	127 Azelaate	179 G-Ketose 6-phosphate
24 2-Furoate	76 p-Hydroxyphenylacetate	128 10-Hydroxydecanoate	180 G-Galactose 6-sulfate
25 3-Acetylacrylate	77 Cysteine sulfinate	129 N-Acetylglutamate	181 Sorbitol6-phosphate
26 Fumarate	78 D-Ethylthiopyrate	130 2,4-D Ethylbromobenzoate	182 2,3-D-Phosphoglycerate
27 Malonate	79 2-Chlorobenzoate	131 O-Carbamylphenylacetate	183 G-Galactosamine 1-phosphate
28 3-Methyl-2-oxobutanoate	80 2,4-D Hydroxypyrimidine-5-carboxylate	132 N-Acetylethionine	184 6-Phosphogluconate
29 Levulinate	81 2-Oxoactanoate	133 Isocitrate	185 Sedoheptulose-7-phosphate
30 Hexanoate	82 Pelargonate	134 Citrate	186 Angiotensin-ate
31 Methylsulfonate	83 a-ketoadipic acid	135 Quinate	187 8-Anilino-1-naphthalene sulfonate
32 2-Hydroxyvalerate	84 Pimelate	136 Phenylacetate	188 N-Acetylglucosamine 1-phosphate
33 Benzoate	85 2-Hydroxy-n-caprylate	137 trans-4-Hydroxy-3-methoxycinnamate	189 N-Acetylglucosamine 6-phosphate
34 Isoisocitrate	86 8-Hydroxyoctanoate	138 Indifenilate	190 N-Acetylglucosamine 6-sulfate
35 1-Aminoethylphosphonate	87 N-Formylaspartate	139 o-Hydroxyhippurate	191 N-Acetylmannosamine
36 2-Aminoethylphosphonate	88 3-Hydroxy-3-methylglutarate	140 G-Ketonate	192 R-Ribose 1,5-diphosphate
37 Isethionate	89 p-Coumarate	141 G-Galactonate	193 N-G-Acetylhexosamine hate
38 D-Ethionate	90 Cumate	142 4-Hydroxy-3-methoxymandelate	194 Fructose 1,6-diphosphate
39 Cyclohexanecarboxylate	91 Terephthalate	143 Syringate	195 R-b-g-Nitrophenylphosphate
40 Mesaconate	92 Phthalate	144 Cysteine S-sulfate	196 G-Ribose hate
41 Itaconate	93 3-Ethoxybenzoate	145 Laurate	197 ProstaglandinE2
42 Citraconate	94 3-(2-Hydroxyphenyl)propionate	146 4-Sulfobenzoate	198 ProstaglandinF2a
43 4-Methyl-2-oxopentanoate	95 3-(4-Hydroxyphenyl)propionate	147 Sebacate	199 Dopamine 5'-monophosphate
44 4-Methylmandelate	96 Phosphoenolpyruvate	148 3-Indolebutyrate	200 D-galactonate
45 4-Acetylbutyrate	97 4-Hydroxy-3-methoxybenzoate	149 Xanthurenate	201 Cholate
46 Heptanoate	98 4-Hydroxyazelate	150 6,8-Thioctate	202 Trehalose 6-phosphate
47 N-Acetylb-alanine	99 Cysteate	151 Ibuprofen	203 Folate
48 G-Ketonate	100 2-Aminoethylphosphonate	152 N-Acetylphenylalanine	204 Adenylosuccinate
49 3-Methylpropionate	101 D-Ethylsuccinate phosphate	153 Benzylsuccinate	205 G-Lysocholate
50 4-Hydroxycaproate	102 4-Chlorophenylacetate	154 N-Benzylsuccinamic acid	206 G-Lysocholate
51 6-Hydroxycaproate	103 G-Isocamphosphonate	155 Mucate	207 Phosphoenol ion
52 Aspartate	104 Dodecanoate	156 3-Indoxyl sulfate	

CE-MS Standards Database

- **Total 788 （Cations 452, Anions 267, Nucleotides 69)**

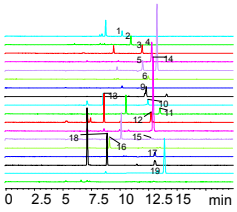
Compound	Carbon	m/z	MT	MT/ MT(IS)	Area	Area/ Area(IS)	S/N	Height
Glyoxylate	2	73	13.834	0.745	19156	0.026	4.515	3906
Propionate	3	73	14.868	0.773	37112	0.033	9.297	7397
Glycolate	2	75	10.099	0.784	59370	0.067	30.675	15631
Crotonate	4	85	15.709	0.817	52279	0.046	19.357	9868
Butyrate	2	87	16.625	0.849	58502	0.072	23.046	7469
Isobutyrate	4	87	17.668	0.853	98571	0.092	33.499	14884
Pyruvate	3	87	11.632	0.736	97905	0.089	10.317	9837
Oxamate	3	88	13.326	0.718	56725	0.025	47.119	13951
Lactate	3	89	14.976	0.796	157643	0.177	71.197	31706
Chloroacetate	2	93	10.198	0.792	17615	0.020	27.051	4739
Tiglate	5	99	20.897	0.844	148596	0.102	28.467	20205
Isovalerate	5	101	14.715	0.932	291806	0.230	25.713	52298
Valerate	5	101	16.847	0.907	166577	0.200	14.756	22547
b-Hydroxybutyrate	4	103	20.683	0.863	247710	0.170	74.924	24253
a-Hydroxybutyrate	4	103	11.647	0.904	164987	0.161	114.899	36482
2-Hydroxyisobutyrate	4	103	16.565	0.846	345230	0.390	260.227	69004
Malonate	1	103	11.008	0.531	18123	0.014	7.250	2338
Glycerate	3	105	15.332	0.815	315357	0.284	176.393	49048

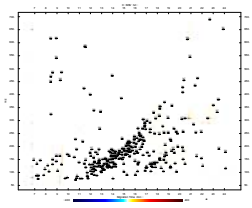
CE-TOFMS (2004)

- CE-MS → CE-TOFMS

- Accurate molecular weight
- 10 times higher resolution
- 2 to 100 times higher sensitivity

- CE-TOFMS Data processing software
  - Metabolome-wide quantitative display
  - Metabolome-wide differential display

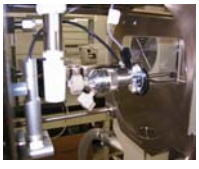




CE-MS/MS

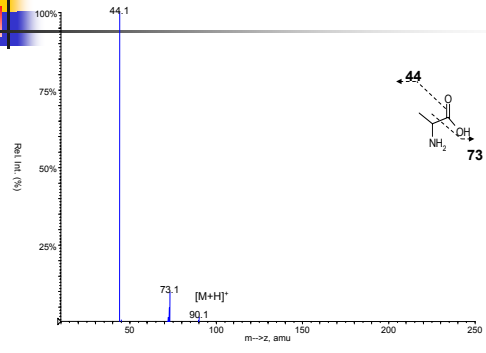
Agilent CE - AB API3000

by Y. Kakazu

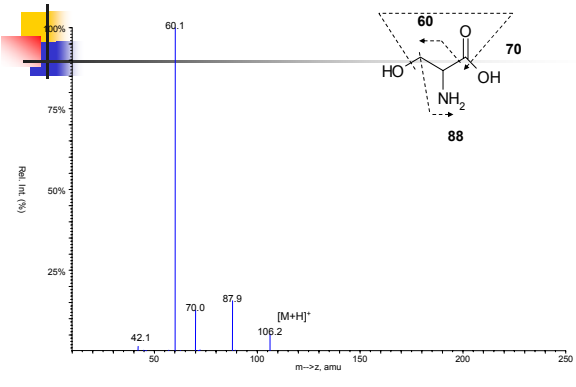


ESI interface

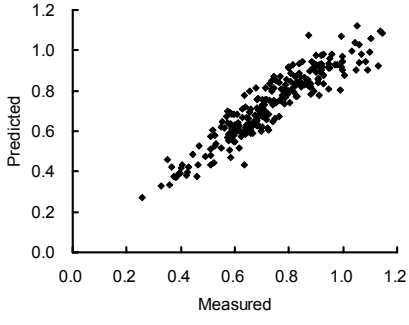
CE/MS/MS Spectrum of L-Ala



CE/MS/MS Spectrum of Ser



CE migration time predicted by ANN



Sugimoto *et al. Analytical Chemistry* **77**:1 (2004)

Analyzing cellular metabolites

- Positively charged molecules – CE/TOFMS
- Negatively charged molecules – CE/TOFMS
- Nucleotides (weakly charged)
  - Pressure-Assisted CE/MS
- Neutral molecules – LC/MS
- Volatile and other molecules – GC/MS

"Metabolome Factory" at Keio-IAB

- 19 x CE systems
- 6 x LC systems
- 2 x GC/MS systems
- 9 x quadrupole MS systems
- 4 x ion-trap MS systems
- 2 x TripleQ MS/MS systems
- 6 x ESI-TOFMS systems
- 1 x Q-TOFMS system
- 1 x NMR

E.coli Project

New Energy and Industrial Technology Development organization (NEDO) of the Ministry of Economy, Trade and Industry of Japan

Institute for Advanced Biosciences

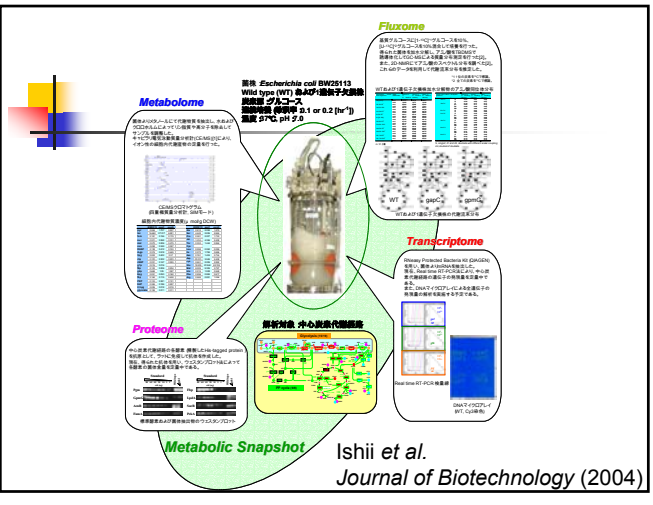
Keio University

Collection of integrated experimental data toward a dynamic model of *E.coli* Metabolism

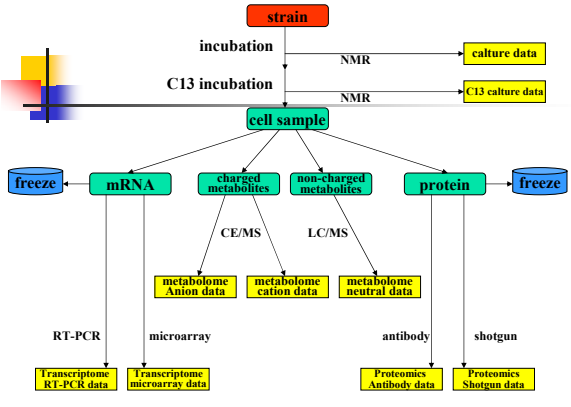
Nobuyoshi Ishii, Tomoya Baba, Tomoyoshi Soga, Akio Kanai, Zhao Jiao, Miki Naba, Li Mai, Satoshi Harada, Aminul Hoque, Yuji Kakazu, Hisashi Kato, Yoshihiro Suga, Kazuharu Arakawa, Katsuyuki Yugi, Yoichi Nakayama, Martin Robert, Takaaki Nishioka, Kazuyuki Shimizu, Hirotada Mori, and Masaru Tomita

Institute for Advanced Biosciences

Keio University



Ishii *et al.*
Journal of Biotechnology (2004)



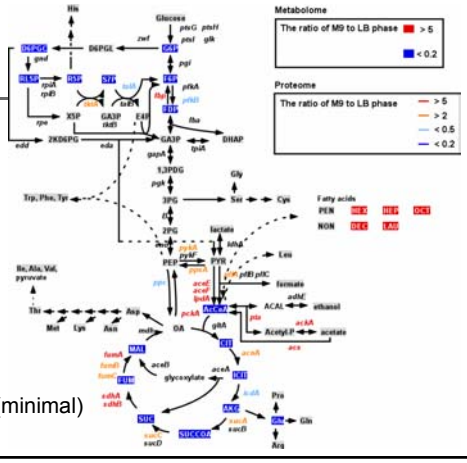
Metabolic Engineering of *E.coli*

Kazuyuki Shimizu

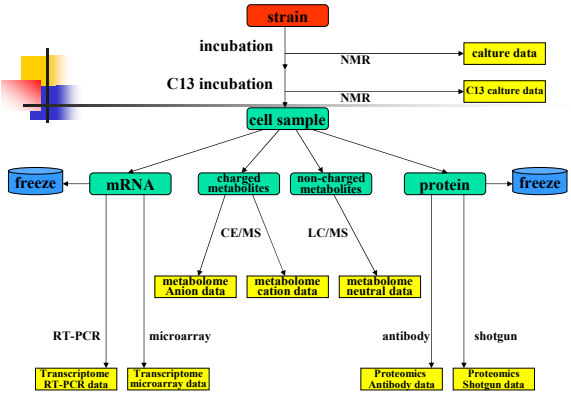
- Metabolic flux distributions (MFDs) with different conditions

Absolute Quantification of *E.coli* Proteome

Naoyuki Sugiyama, Takeshi Masuda, Kousaku Shinoda, Nozomu Yachie, Yuji Kakazu, Martin Robert, Hirotada Mori, Takaaki Nishioka, Tomoyoshi Soga and Masaru Tomita

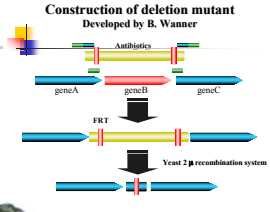


LB(rich) vs M9(minimal)



E.coli Genome Engineering

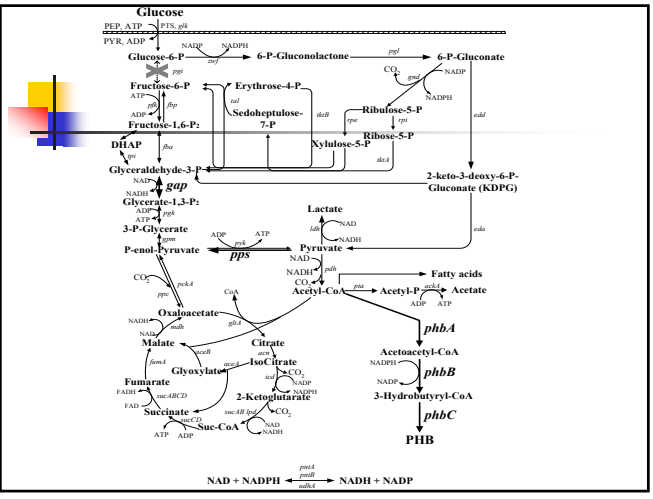
- *E.coli*
  - 4200 gene clone library
  - Single gene destructive mutants



Hirotada Mori

Single Deletion Mutant Library

"*E.coli* KO Collection"



Computer modeling of *E.coli* metabolism

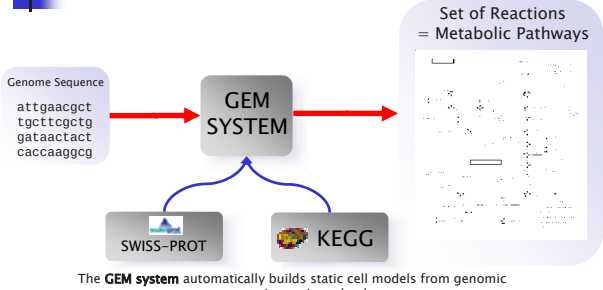
Towards cell-wide metabolic pathways

- Top-town: Functional genomics
- Bottom-up: Metabolomics
- Integration: Bioinformatics

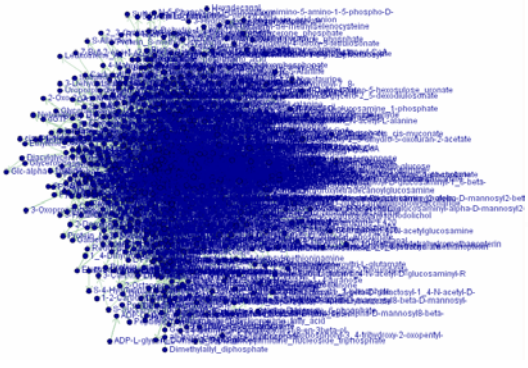
GEM System:

Automatic Conversion of Genome Sequences into E-Cell models

Automatic construction of metabolic pathways



The **GEM system** automatically builds static cell models from genomic sequences using various databases.

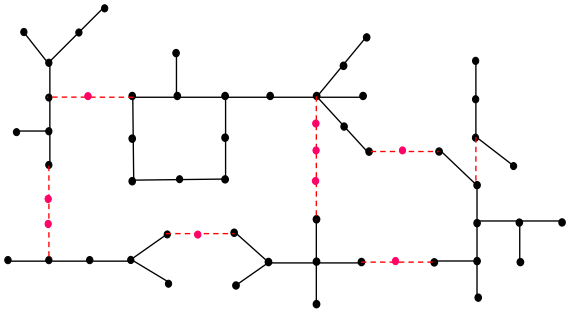


Escherichia coli K12 MG1665

Metabolites： 940、 Enzymes： 1178、 Reactions： 1179

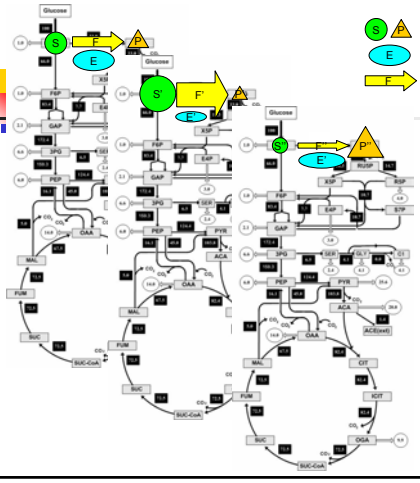
index.html

Construction of Whole Cell Models



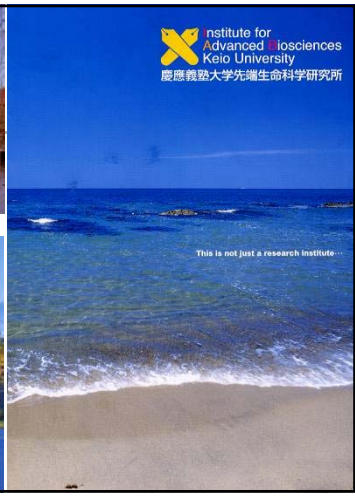
Towards "Complete Metabolome"

- Top-town: Functional genomics
- Bottom-up: Metabolomics
- Integration: Bioinformatics



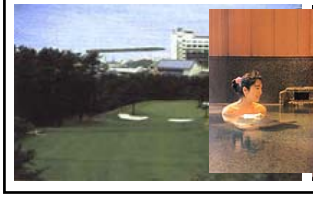
For each enzymatic reaction,
- **flux**
- **quantity of enzyme**
- **quantity of substrate, product, cofactors**

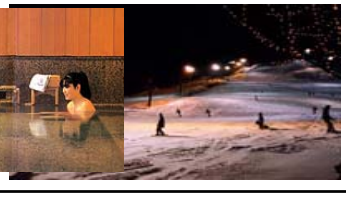
→ parameter fitting by GA



鶴岡市

Tsuruoka City





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1st International Conference on Metabolomics

- June 20-23, 2005
- Tsuruoka, Japan
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