Supplementary information – SnapShot: Basic numbers for cell biology:

Cell Sizes:

1. Bacteria (*E. coli*): ~1 μm diameter (BNID 100002)

Ref: Nelson DE, Young KD. Penicillin binding protein 5 affects cell diameter, contour, and morphology of Escherichia coli. J Bacteriol. 2000 PMID <u>10692378</u>

Measurement Method: Cell measurements and analyses were performed with Image Pro. Cell diameters of individual cells were calculated by measuring the cross-sectional area and dividing that value by the cell length.

Comments: The derived diameters of 92% of untreated wild-type E. coli CS109 cells clustered between 1.0 and 1.1 µm (Fig. 3A)

~2.5 µm length (BNID 103713)

Ref: Grossman N, Ron EZ, Woldringh CL. Changes in cell dimensions during amino acid starvation of Escherichia coli. J Bacteriol. 1982 PMID 6749809

Measurement Method: Electron micrographs were projected at a final magnification of about 15,000x to 20,000x, and cell length and cell diameter were measured. At least 150 cells were measured for each sample, and dimensions were calculated according to a calibration grid which was photographed at the same magnification.

Comments: Value is mean of E. coi B ilvA, thyA, cells grown on glycerol and glucose during exponential phase, Mean doubling time 57 min.

 $\sim 1.1 \ \mu m^3 \ in \ volume \ 0.44-1.79 \ \mu m^3 \ (BNID \ 100004)$

Ref: Kubitschek HE, Friske JA. Determination of bacterial cell volume with the Coulter Counter. J Bacteriol. 1986 Dec168(3):1466-7. Table link-http://tinyurl.com/cphmpq PMID 3536882

Measurement Method: Coulter Counter analyzer system **Comments**: value is average of 10 measurements in table 1

2×10⁷ cell concentration for culture with OD600 of 0.1 (BNID 103625)

Ref: Sezonov G, Joseleau-Petit D, D'Ari R. Escherichia coli physiology in Luria-Bertani broth. J Bacteriol. 2007 Fig. 1a PMID 17905994

Measurement Method: The widely used wild-type E. coli K-12 strain MG1655 was chosen for this study. An overnight culture in Luria-Bertani broth was diluted 5,000-fold in 250 ml fresh medium in a 1-liter Erlenmeyer flask and cultivated in a shaking water bath (180 rpm) at 37°C. The OD600 and number of cells per milliliter were monitored.

Comments: Note that cell concentration of a different strain with OD600 of 0.1 is 10^8 cells/ml, greater by a factor of 5 (see BNID 100985). The optical density of yeast and bacterial cultures is due to light scattering, not absorption. Therefore it is sensitive to the geometry of the detector relative to the cuvette, as well as the size and shape of the cells.

2. Yeast (Saccharomyces cerevisiae): ~5 μm diameter (BNID 103896)

Ref: Teerapatr Srinorakutara, Determination of yeast cell wall thickness and cell diameter using new methods, Journal of Fermentation and Bioengineering Volume 86, Issue 3, 1998, Pages 253-260

Measurement Method: The cell diameters were measured using a Coulter counter and an image analyser. Micromanipulation was used for measuring the bursting strength of individual yeast cells. The basic principle of this technique is the capture and squeezing of a single cell between parallel surfaces.

Comments: S. cerevisiae Yg grown in YEPG medium at 60% DO₂.

~70 µm³ in volume (BNID 100452) **Ref**: Roskams and Rodgers, LabRef

3. Volume of mammalian (HELA) cell 4400-5000 μm³ (BNID 103719)

Ref: Cohen LS, Studzinski GP. Correlation between cell enlargement and nucleic acid and protein content of HeLa cells in unbalanced growth produced by inhibitors of DNA synthesis. J Cell Physiol. 1967 Jun6 9(3):331-9. PMID 4230858

Measurement Method: Cells allowed to enter logarithmic phase. Cell number was obtained with a Coulter counter, while cell sizing was performed with the automatic particle size distribution analyser Model J Electronic Co., Hialeah, Fla.

Comments: Please note-BNID 103725 gives HeLa cell volume range of 760-2730 um³

Human erythrocyte 97 μm³ in volume (BNID 101711)

Ref: Frank, A. O., C. J. Chuong, et al. (1997). "A finite-element model of oxygen diffusion in the pulmonary capillaries." J.Appl.Physiol 82(6): 2036-2044. PMID <u>9173974</u>

COS-7 cell 2016+-209 µm³ (BNID 101666)

Ref: Bohil et al. Myosin-X is a molecular motor that functions in filopodia formation. PNAS 2006. 103(33): pp.12411-12416 PMID 16894163

Comments:value is found in supplemental figure 7. The COS cell line was obtained by immortalizing a CV-1[1] cell line derived from kidney cells of the African green monkey.

Human alveolar macrophage cell 4990+-174 µm³ (BNID 103566)

Ref: Krombach F, Münzing S, Allmeling AM, Gerlach JT, Behr J, Dörger M. Cell size of alveolar macrophages: an interspecies comparison. Environ Health Perspect. 1997 Sep105 Suppl 5:1261-3. Table 2 PMID 9400735

Measurement Method: Morphometric analysis of AM was performed using a flow cytometer that generates volume signals based on the Coulter-type measurement of electrical resistance.

Perikaryon of purkinje cell in human cerebellum 13700 µm³ (BNID 103180)

Ref: Andersen BB, Gundersen HJ, Pakkenberg B. Aging of the human cerebellum: a stereological study. J Comp Neurol. 2003 Nov 17466(3):356-65. *PMID* 14556293

Measurement Method: stereological tools designed for estimating mean particle volume: The nucleator and the rotator.

Comments: Cerebella from 19 normal Caucasian males, ages 19-84 years, were studied. The perikaryon is also known as the cell body.

Organelles and cell constituents:

4. Fraction of Budding yeast nucleus volume out of total cell volume ~7% (BNID 104708)

**Ref: Jorgensen P, Edgington NP, Schneider BL, Rupes I, Tyers M, Futcher B. The size of the nucleus increases as yeast cells grow. Mol Biol Cell. 2007 Sep18(9):3523-32. Table link - http://tinyurl.com/ncgbno PMID 17596521

Measurement Method: Morphometry

Comments: Analysis of mutant yeast strains spanning a range of cell sizes revealed that the ratio of average nuclear volume to average cell volume was quite consistent, with nuclear volume being approximately 7% that of cell volume. See Nuclear volume of 2.9 um³ BNID 100447

HeLa cell nucleus in cervix tissue $\sim 400 \ \mu m^3$ in volume (BNID 101402)

Ref: Maul GG, Deaven L. Quantitative determination of nuclear pore complexes in cycling cells with differing DNA content. J Cell Biol. 1977 Jun73(3):748-60.3. Table 1 Table link – http://tinyurl.com/58x8ay PMID 406262

Measurement Method: HeLa, mouse L, and CHO cells were grown in suspension culture. Their nuclei appeared mostly round. The long and short axes of these nuclei were averaged, and the surface and volume were calculated as if the nuclei were spheres.

5. Cell membrane thickness ~4-10 nm

(BNID 103944) 5-10 nm for E. coli

Ref: EL-Hag, A. Jayaram, S.H. Effect of biological cell size and shape on killing efficiency of pulsed electric field, Dielectric Liquids, 2008. ICDL 2008. IEEE International Conference on Volume, Issue, June 30 2008-July 3 2008 Page(s):1 - 4 Table 2

(BNID 101276) 4.1 nm for spinach

Ref: Proton-powered turbine of a plant motor. Seelert H et al. Nature. 2000 May 25;405(6785):418-9PMID 10839529

Primary Source: Hine, Robert. "Membrane." The Facts on File Dictionary of Biology. 3rd ed. New York: Checkmark, 1999: 198.

Measurement Method: atomic force microscopy

(BNID 103948) 7.5 nm for vertebrate erythrocyte

Ref: Jaspard F, Nadi M, Rouane A. Dielectric properties of blood: an investigation of haematocrit dependence. Physiol Meas. 2003 Feb24(1):137-47. PMID <u>12636192</u> **Primary Source:** Aim'e-Genty N 1999 Le sang Dictionnaire Encyclop'edique (Paris: Vuibert)

6. "Average" protein diameter ~4 nm (BNID 101827)

Ref: "Physical Biology of the Cell", Rob Phillips, Jane Kondev and Julie Theriot (2009). Page 26

Comments: See Stokes radius of Casein 2.9-3.7 nm (BNID 103862), Radius of gyration of myoglobin 1.52 nm (BNID 103782), Radius of gyration of ribonuclease1.43 nm (BNID 103781), Radius of gyration of conalbumin 2.95 nm (BNID 103737), albumin diameter 3 nm (BNID 103736), insulin receptor diameter 6.5 nm (BNID 103212), Diameter of the LacY transporter ~6 nm (BNID 102929)

Properties of casein, BSA, and lysozyme Table link - http://tinyurl.com/cu28na (BNID 103880)

Ref: Pim van Hee, Selective recovery of micrometer bioparticles using aggregation and air flotation, thesis, 2006, Department of Biotechnology, Delft University of Technology The Netherlands

Comments: Radii of casein, BSA, and lysozyme 2.9-3.7, 3.48, and 1.8 nm, respectively

Radius of gyration of ribonuclease 1.43 nm (BNID 103781)

Ref: Yaoqi Zhou, Salt Effects on Protein Titration and Binding, J. Phys. Chem. B, 1998, 102 (51), pp 10615–10621 table 1

Measurement Method: X ray crystallography

Comments: Estimated hydrated protein radius is 1.71 nm

Radius of gyration of myoglobin 1.52 nm (BNID 103782)

Ref: Yaoqi Zhou, Salt Effects on Protein Titration and Binding, J. Phys. Chem. B, 1998, 102

(51), pp 10615–10621 table 1

Measurement Method: X ray crystallography

Comments: Estimated hydrated protein radius is 1.84 nm

7. Water molecule diameter ~ 0.28 nm (BNID 103723)

Ref: D'Arrigo JS. Screening of membrane surface charges by divalent cations: an atomic representation. Am J Physiol. 1978 Sep235(3):C109-17 PMID 696813

Comments: Other sources give value of 2.63 Angstrom

Concentrations:

8. Concentration of 1 nM in a cell the volume of *E. coli* is ~ 1 molecule/cell (BNID 102068)

**Ref: Calculated according to cell volume in Primary Source, please see Measurement Method **Primary Source: Kubitschek HE, Friske JA. Determination of bacterial cell volume with the Coulter Counter. J Bacteriol. 1986 Dec168(3):1466-7. Table link-http://tinyurl.com/cphmpq PMID 3536882

Measurement Method: Calculated using Avogadro's constant according to one cell volume=1E-12 cm^3=1E-15 liter (Average of 10 measurements in table from Primary source, BNID 100004). Calculated using Avogadro's constant according to one cell volume=1E-12 cm^3=1E-15 liter. 1 particle/1E-15liter=X particles/liter>>X=1E15 particles/liter 1E15(particles/liter)/6E23(particles/mole)=1.666E-9M=~1 nM.

Concentration of ~ 0.1 pM in a mammalian (HeLa) cell is ~ 1 molecule/cell (BNID 104519) **Ref**: Calculated according to cell volume, please see Measurement Method Measurement Method: Calculated using Avogadro's constant according to one cell volume=5000 um^3=5E-12 liter (BNID 103719). 1 particle / 5E-12 liter = X particles/liter >> X = 2E11 particles/liter 2E11 (particles/liter)/6E23 (particles/mole) = $\sim 3E-13$ M= ~ 0.1 pM. Thus 1 mM= $\sim 10^{10}$ molecules per cell. Note: 3E-13 M and 1E-13 M are on same order of magnitude, allowing approximation.

9. Characteristic concentration for a signaling protein ~10 nM-1µM (BNID 103954)

> Ref: S Legewie, H Herzel, H Westerhoff, and N Bluthgen. Recurrent design patterns in the feedback regulation of the mammalian signalling network. Molecular Systems Biology. 2008. 4:190 Table link - http://tinyurl.com/5sm66v PMID 18463614

Primary Source: See table for primary sources

Measurement Method: The Table gives a characteristic range of signaling proteins 10000-1000000 molecules per cell. Calculated using Avogadro's constant according to one cell volume (mammalian cell culture volume BNID 100434) ~2E-15cm^3 =2E-12liter. 2E-12X6E23=~1E12 particles per cell with concentration of 1 Molar. Concentration of 0.1-1 uM would give 1e4-1e6 molecules per cell.

Comments: Table gives Number of various signaling protein molecules in one cell. This table can be found in the supplementary information section of the above-mentioned reference. 0.4 µM concentration of Ras in HeLa cells (BNID 100853)

Ref: Fujioka et al., Dynamics of the Ras/ERK MAPK cascade as monitored by fluorescent probes. J Biol Chem. 2006 Mar 31281(13):8917-26 PMID 16418172

Measurement Method: FRET (Fluorescence Resonance Energy Transfer)probe

10. Water content: ∼70% of cell

E. coli 74%, (BNID 100044)

Ref: Jerry W. King, Gary R, Supercritical fluid technology in oil and lipid chemistry, 1996, American Oil Chemists' Society, pp. 303

Yeast, 68% (BNID 103689)

Ref: Jerry W. King, Gary R, Supercritical fluid technology in oil and lipid chemistry, 1996, American Oil Chemists' Society, pp. 303

Rat Alveolar macrophage 67% (BNID 103960)

Ref: Banks MA, Porter DW, Pailes WH, Schwegler-Berry D, Martin WG, Castranova V. Taurine content of isolated rat alveolar type I cells. Comp Biochem Physiol B. 1991 100(4):795-9. Table link - http://tinyurl.com/cybwjc PMID 1782762

Measurement Method: Rat alveolar type I cells were isolated by enzymatic digestion and purified by centrifugal elutriation and specific surface adsorption. The identity of the harvested cells was confirmed using electronic cell sizing and transmission electron microscopy. Calculated according to cell volume of 1456 um³=1.456e-12 liter and water content of 0.97 ul/10⁶ cells=0.97*10⁻¹² Liter per cell

Empirical elemental formula for biomass of E. coli C:H_{1.77}:O_{0.49}:N_{0.24} (BNID 101800) **Ref**: von Stockar, U. Liu, J. Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth. 1999. Biochim Biophys Acta. 1412(3) p.198 PMID 10482783

Comments: This means that for every mole of carbon, there is 1.77 moles of hydrogen, 0.49 moles of oxygen and 0.24 moles of nitrogen. From this the carbon content of a cell is about 48% of the dry weight

11. Composition of dry weight of an E. coli: ~55% protein, 20% RNA, 10% Lipids, 15% others (BNID 101436)

Ref: Neidhardt F.C. Escherichia coli and Salmonella: Cellular and Molecular Biology. Vol 1. pp. 14, ASM Press 1996. Table 1Table Link - http://tinyurl.com/cr3y57

Comments: General information on quantities of macromolecules per cell

Composition of dry weight of an S. cerevisiae: protein 40% fiber 30% RNA 10% ashes 4% lipids 1% others 15% (BNID 104157)

Ref: Yamada EA, Sgarbieri VC. Yeast (Saccharomyces cerevisiae) protein concentrate: preparation, chemical composition, and nutritional and functional properties. J Agric Food Chem. 2005 May 1853(10):3931-6 Table link - http://tinyurl.com/nl8z68 PMID 15884819 Measurement Method: Water content, ashes, and crude protein (N X5.8) were determined according to the AOAC (ref 15) procedures. Total lipids were determined according to the Bligh and Dyer method (16). Soluble and insoluble fibers were quantified by treating the sample first with proteolytic enzymes (pepsin/pancreatin) to digest sample protein, followed by filtration to retain the insoluble fiber and precipitation of the soluble fiber from the filtrate with ethanol.

12. Number of proteins in an *E. coli* cell 2.35×10⁶ (depending on growth rate) (BNID 102990)

**Ref: Neidhardt F.C. Escherichia coli and Salmonella: Cellular and Molecular Biology. Vol 1.

*ASM Press 1996. table link- http://tinyurl.com/cr3y57

Measurement Method: Calculated for an average cell of E. coli B/r in balanced growth at 37 degrees celsius in aerobic glucose minimal medium with a 40 minute mass doubling time. This cell is 44% through its division cycle.

Calculated concentration of total protein in *E. coli* cell ~4 mM (BNID 104726)

Ref: Calculated according to Primary Source. Please see Measurement Method Neidhardt F.C. Escherichia coli and Salmonella: Cellular and Molecular Biology. Vol 1. ASM Press 1996. table link- http://tinyurl.com/cr3v57

Measurement Method: Calculated for an average cell of E. coli B/r in balanced growth at 37 degrees celsius in aerobic glucose minimal medium with a 40 minute mass doubling time. This cell is 44% through its division cycle. Calculated from Protein number of 2.35e6 (See BNID 102990 and table link in Primary Source) and assuming cell volume of ~1um^3=1e-15liter (BNID 10004) and avogadro's constant=6e23 particles/mole (BNID 101907). (2.35e6proteins/cell)/[(6e23proteins/mole)*(1e-15liter/cell)]=~4e-3M

Total observed intracellular metabolite pool in E. coli 300 mM (BNID 104678)

Ref: Bennett BD, Kimball EH, Gao M, Osterhout R, Van Dien SJ, Rabinowitz JD. Absolute metabolite concentrations and implied enzyme active site occupancy in Escherichia coli. Nat Chem Biol. 2009 Aug5(8):593-9 Table link - http://tinyurl.com/m8rymt PMID 19561621

Measurement Method: Researchers quantified metabolites by Liquid chromatography-mass spectrometry (LC-MS/MS) using an isotope ratio-based approach (8). As isotope-labeled standards for many metabolites are not available, they used uniformly 13C-labeled glucose medium to label the intracellular metabolome of E. coli (25). This enabled the use of commercially available unlabeled compounds as internal standards.

Comments: The total observed intracellular metabolite pool was approximately 300 mM. A small number of metabolites dominate the metabolome on a molar basis, with glutamate being the most abundant.

Energetics:

ΔG needed to achieve order of magnitude ratio of concentrations: ~6 kJ/mole
 -5.7 kJ/mole (BNID 103912)

Ref: Nelson & Cox, Lehninger's Principles of Biochemistry 4th Edition, 2004, WH Freeman Table 6-4 pp.195

Measurement Method: Delta G=-RTLn(Keq). Calculating for R=8.315, T=298, Keq=10, Delta G=5705.5 J/mole

Comments: Approximately 2kT=Approximately 60meV. Calculating RT=kT/(Avogadro's number)

14. Energetic contribution of a hydrogen bond: ~6-20 kJ/mole (BNID 103914, 103913, 101067, 101069, 101068, 101070)

9 kJ/mole Strength of hydrogen bond in water (BNID 103914)

Ref: van der Spoel D, van Maaren PJ, Larsson P, Tîmneanu N. Thermodynamics of hydrogen bonding in hydrophilic and hydrophobic media. J Phys Chem B. 2006 Mar 9110(9):4393-8. PMID 16509740

Measurement Method: An extensive series of over 400 molecular dynamics simulations with an aggregate length of over 900 ns was analyzed using an analysis technique in which hydrogen bond (HB) breaking is interpreted as an Eyring process, for which the Gibbs energy of activation ΔG can be determined from the HB lifetime.

15. ΔG of ATP hydrolysis under physiological conditions in *E. coli*~50 kJ/mole (BNID 101701) **Ref**: Quang Hon Tran, Gottfried Unden. Changes in the proton potential and the cellular energetics of Escherichia coli during growth by aerobic and anaerobic respiration or by fermentation. European Journal of Biochemistry. 1998. 251(1-2) pp. 538-543 PMID 949233 **Measurement Method**: calculated from the equation: delta G (Phos) = delta G (at standard conditions) + RT $\ln([ATP]/[ADP][Pi])$, where R is the gas constant, T = temperature, and - 30.5 kJ/mol was used for delta G at standard conditions.

Comments: The number has been converted from positive to negative so that it describes the Gibbs free energy of hydrolysis as opposed to the phosphorylation potential. The E. coli was growing in the presence of oxygen. See reference for more details.

16. Number of ATP molecules required to make an *E. coli* cell on minimal medium and glucose under Aerobic conditions $\sim 10^{10}$ ATP (BNID 101981)

Ref: Farmer, I.S. and Jones, C.W. The energetics of Escherichia coli during aerobic growth in continuous culture. (1976) Eur. J. Biochem. 67(1):115-22. PMID 786616

Measurement Method: YATP(max) was determined by measuring the rate of oxygen consumption(QO2) and growth rate(D), and calculating the value of mol ATP formed per mol oxygen (N) and the energetic maintenance cost (M). The equation QO2 = D/YATP(max)*N + M/N was used.

Comments: This number is calculated from the value for YATP(max) of 13.9 in the reference. YATP(max) is the number of grams of cells (dry weight) produced per mol of ATP corrected for cellular maintenance energy costs. 1/13.9 gives 0.0719 mol of ATP per gram of cells. One E.coli cell weighs 0.28pg (dry wt), so there are 3.57 trillion cells/gram. 0.0719*6.02E23/(3.57E12 cells/g (dry wt)) gives 12.1 billion ATP/cell.

Number of ATP molecules to make an *E. coli* cell on minimal media and glucose under anaerobic conditions 1.64×10^{10} ATP (BNID 101983)

Ref: Hempfling, W. and Mainzer, S. Effects of Varying the Carbon Source Limiting Growth on Yield and Maintenance Characteristics of Escherichia coli in Continuous Culture. (1975) J. Bacteriol. 123(3): 1076-1087. PMID 169226

Comments: This number is calculated from the value for YATP(max) of 10.3 in the reference. YATP(max) is the number of grams of cells (dry weight) produced per mol of ATP corrected for cellular maintenance energy costs. 1/10.3 gives 0.0971 mol of ATP per gram of cells. One E.coli cell weighs 0.28pg (dry wt), so there are 3.57 trillion cells/gram. 0.0971*6.02E23/(3.57E12 cells/g (dry wt)) gives 16.4 billion ATP/cell.

ATP requirement for growth on acetate and minimal media under aerobic conditions 2.48×10^{10} ATP (BNID 101982)

Ref: Farmer, I.S. and Jones, C.W. The energetics of Escherichia coli during aerobic growth in continuous culture. (1976) Eur. J. Biochem. 67(1):115-22. *PMID* 786616

Measurement Method: YATP(max) was determined by measuring the rate of oxygen consumption(QO2) and growth rate(D), and calculating the value of mol ATP formed per mol oxygen (N) and the energetic maintenance cost (M). The equation QO2 = D/YATP(max)*N + M/N was used.

Comments: This number is calculated from the value for YATP(max) of 7.1 in the reference. YATP(max) is the number of grams of cells (dry weight) produced per mole of ATP corrected for cellular maintenance energy costs. 1/7.1 gives 0.1408 mol of ATP per gram of cells. One E.coli cell weighs 0.28pg (dry wt), so there are 3.57 trillion cells/gram. 0.1408*6.02E23/(3.57E12 cells/g (dry wt)) gives 24.8 billion ATP/cell.

ATP requirement for growth and Yatp under different nutritional conditions Table Link - http://tinyurl.com/qjojft (BNID 101637)

Ref: Byung Hong Kim, Geoffrey Michael Gadd. Bacterial Physiology and Metabolism. Cambridge University Press.

Primary Source: Dawes, Edwin A. Microbial Energetics. Chapman and Hall: New York, NY. 1986. It appears to be based on a table from a chapter of Microbial Biochemistry - see Stouthamer, A.H. The search for correlation between theoretical and experimental growth yields. (1979) Int. Rev. Biochem. Microb. Biochem. 21:1-47 ed. J.R. Quayle. University Park Press.

Comments: After consulting primary sources, it appears that for columns E and F, and possibly G, the energy requirement for mRNA turnover was already included in the RNA cost, and so has been counted twice in this table. This would reduce the "Total" by 1.39 mmol of ATP for these columns, which would cause a very slight change in Yatp.

Diffusion and catalysis rate

17. Diffusion coefficient of dextran (40kDa) in Drosophila cytoplasm 17.6+-1.8 μm²/sec (BNID 100198)

Ref: Gregor T, Bialek W, de Ruyter van Steveninck RR, Tank DW, Wieschaus EF. Diffusion and scaling during early embryonic pattern formation. Proc Natl Acad Sci U S A. 2005 Dec 20102(51):18403-7. PMID <u>16352710</u>

Measurement Method: Fluorescent dextran injected into Drosophila embryo, confocal imaging, and fitting with 3D diffusion model

7.7+-2.5 µm²/sec GFP Diffusion coefficient in E. coli cytoplasm (BNID 100193)

Ref: Elowitz MB, Surette MG, Wolf PE, Stock JB, Leibler S. Protein mobility in the cytoplasm of Escherichia coli. J Bacteriol. 1999 Jan181(1):197-203. PMID <u>9864330</u>

Measurement Method: FRAP and photoactivation

Time for protein to diffuse across E. coli cell ~0. 1 sec (BNID 104667)

Ref: Calculated manually. Please see measurement method.

Measurement Method: According to equation: t diffusion= \sim X^2/2D. Taking X, distance to be traveled, as 1um and D, diffusion coefficient, as 100um^2/sec. D calculated from D=KBT/6/pi/ η /R where R=2.5nm, typical protein diameter. KB=Boltzmann's constant, η (eta)=viscousity of the medium, taken as 0.001 Pa*sec for water at \sim 300 Kelvin, T=temperature in degrees Kelvin, pi= \sim 3.14. (1.380 6504(24)×10^-23 Kgm^2 sec^-2K^-1×300K)/(6×3.14×0.001Kgm^-1sec^-1×2.5×10^-9 m)=8.8×10^-11m^2/sec=88um^2sec= \sim 100um^2/sec

Comments: Diffusion coefficient of 100 um^2/sec refers to water. In cytoplasm D is smaller than $100 \text{um}^2/\text{sec}$ as there are solutes, in the range of 5-15 um^2/sec (BNIDs 100193, 100198). Taking D as $10 \text{ um}^2/\text{sec}$, actual time of diffusion would be $(1 \text{um})^2/(2 \times 10) \text{um}^2/\text{sec} = 0.1$ sec

~5 sec for hemoglobin to traverse erythrocyte (BNID 104677)

Ref: Calculated according to erythrocyte diameter. Please see Measurement Method **Measurement Method:** Calculated with equation d^2=2Dt where d=distance to pass, length of erythrocyte, 10um. D of hemoglobin in erythrocyte cytoplasm is lower than in water due to presence of solutes, in the range of 5-15 um^2/sec (BNIDs 100193, 100198). Taking D as 10 um^2/sec, actual time of diffusion would be $(10\text{um})^2/(2\times10)\text{um}^2/\text{sec} = 5$ sec BNID 104106 for diffusion time in water medium

D (metabolite) $\sim 500 \, \mu m^2 / sec$

Diffusion coefficient of sucrose in water 520 µm²/sec (BNID 100614)

Ref: S. Vogel Life's Devices (1988)

Diffusion coefficient of glucose in water 600 µm^2/sec (BNID 104089)

Ref: Stein, Wilfred D., Channels, carriers and pumps An introduction to Membrane Transport, 1990 Academic press inc. Harcourt Brace Jovanovich, Publishers pp.32 Table link - http://tinyurl.com/prfosd

Primary Source: R. Hober (1967) "Physical Chemistry of cells and tissues" Churchill, London AND Daniels and Alberty (1961) "Physical Chemistry" Wiley, New York

Comments: Table gives Molecular/Ionic weight and Diffusion coefficients of various substances in specified temperatures

18. Diffusion limited on-rate for a characteristic protein ~10⁸-10⁹ 1/sec/Molar (BNID 103916)

**Ref: Nelson & Cox, Lehninger's Principles of Biochemistry 4th Edition, 2004, WH Freeman & Co, pp.207 Table link - http://tinyurl.com/cgoqnu

Comments: There is an upper limit to Kcat/Km, imposed by the rate at which E and S can diffuse together in an aqueous solution. This diffusion controlled limit is 10^8 to 10^9 M 1 xs 1 , and many enzymes have a kcat/Km near this range

Division, Replication, transcription, translation rates

19. Exponential cell cycle time:

E. coli ~30 minutes (BNID 103890)

Ref: Bremer, H., Dennis, P. P. (1987) Modulation of chemical composition and other parameters of the cell by growth rate. Neidhardt, et al. eds. Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology, 1st ed. chapter 96, Table 2 pp.1530-1 Comments: Table gives Protein/mass, RNA/mass, DNA/mass, cell no./mass, protein/genome, RNA/genome, origins/genome, protein/origin and more parameters at 5 different doubling times

Yeast: ~100 minutes (BNID 100270)

Ref: JR Warner, The economics of ribosome biosynthesis in yeast, Trends Biochem Sci. 1999 Nov24(11):437-40. PMID 10542411

Comments: Can reach ~70 minutes under ideal conditions (see BNID 101747)

Population doubling time, percent budded cells, and mean cell volume for different batch culture media 71-477 min (BNID 101747)

Ref: Tyson CB, Lord PG, Wheals AE. Dependency of size of Saccharomyces cerevisiae cells on growth rate. J Bacteriol. 1979 Apr138(1):92-8. PMID <u>374379</u>

Measurement Method: The mean size and percentage of budded cells of a wild-type haploid strain of Saccharomyces cerevisiae grown in batch culture over a wide range of doubling times (tau) have been measured using microscopic measurements and a particle size analyzer. **Comments:** Shortest generation time 71 min on YEP+fructose medium Longest generation time 477 min on EMM+acetate+phthalate

Human cell line (Hela): 16.2 h (BNID 103804)**Ref**: Kumei Y, Nakajima T, Sato A, Kamata N, Enomoto S. Reduction of G1 phase duration and enhancement of c-myc gene expression in HeLa cells at hypergravity. J Cell Sci. 1989 Jun93 (Pt 2):221-6. PMID <u>2693468</u>

Measurement Method: Hypergravity cell culture, [3 H] thymidine incorporation, Cell cycle analysis, RNA blot hybridization (Northern analysis)

Comments: Research checked difference between HeLa cells in hypergravity conditions and control.

Doubling time of cancerous cell lines 17-80 hours (BNID 100685)

Ref: http://dtp.nci.nih.gov/docs/misc/common files/cell list.html

Measurement Method: In vitro

Comments: Information on generation time, in hours, of the NCI set of 60 cancerous cell lines. Range 17-80 hours

Doubling time of human cancerous cell lines 13-80 hours (BNID 103994) *Table link - http://tinyurl.com/clejlo*

Ref: Patent - JOINT USE OF SULFONAMIDE BASED COMPOUND WITH ANGIOGENESIS INHIBITOR-OWA, OZAWA, and SEMBA 20.06.2007 link - http://tinyurl.com/cd9eko page 38 table 1

Measurement Method: Cells of these cell lines were seeded in 96-well microplates (flat-bottomed) (50 Pl/well) at the cell numbers indicated in Table 1. After 24 hours, a 3-fold dilution series of each compound was added (50 Pl/well). After 72 hours, WST-8 (10 Pl/well) was further added and the absorbance at 450 nm was measured. Additional information under table.

Comments: Information on generation time, in hours, of 36 cancerous cell lines. Range 13-80 hours.

20. Average replication rate of T7 bacteriophage DNA in *E. coli* 220+-80 nucleotides/sec (BNID 103995)

Ref: Lee JB, Hite RK, Hamdan SM, Xie XS, Richardson CC, van Oijen AM. DNA primase acts as a molecular brake in DNA replication. Nature. 2006 Feb 2439(7076):621-4. Supplementary information. PMID 16452983

Measurement Method: in vitro, single molecule measurements. The 5' end of one strand of a 48.5 kilobase (kb)-long duplex lambda phage DNA molecule is attached to the bottom surface of a glass flow cell using a biotin–streptavidin linker. The opposite 3' end bears a digoxigenin moiety and is linked to a bead that is 2.8 microm in diameter and coated with anti-digoxigenin antibody

Comments: These values are in agreement with those observed in a previously published single-molecule experiment: Wuite, G. J. L., Smith, S. B., Young, M., Keller, D. & Bustamante, C. Single-molecule studies of the effect of template tension on T7 DNA polymerase activity. Nature (London) 404, 103-6 (2000). PubMed ID 10716452, that give polymerization rates of over 100 bases/sec for T7 DNA

DNA synthesis rate of *E. coli* DNA polymerase III holoenzyme ~750nts/sec (BNID 104120) *Ref:* Kelman Z, O'Donnell M. DNA polymerase III holoenzyme: structure and function of a chromosomal replicating machine. Annu Rev Biochem. 1995 64:171-200 PMID 7574479 *Primary Source:* Studwell PS, O'Donnell M. Processive replication is contingent on the exonuclease subunit of DNA polymerase III holoenzyme. J Biol Chem. 1990 Jan 15265(2):1171-8 PMID 2153103

Measurement Method: In vitro. The reconstitution approach was taken to study which subunits of the heterotrimer core polymerase (alpha, epsilon, theta) participate in the highly processive replication of long DNA templates by DNA polymerase III holoenzyme (holoenzyme).

Comments: This value is consistent with the rate of fork movement in E. coli. This rapid rate results from the high processivity of holoenzyme, which extends a chain for several thousand nucleotides without dissociating from the template even once

DNA replication rate in human 33 nts/sec (BNID 104136)

Ref: Fangman WL, Brewer BJ. A question of time: replication origins of eukaryotic chromosomes. Cell. 1992 Oct 3071(3):363-6 PMID 1423601

Measurement Method: Replication fork speed of ~ 2000 base pairs/min=2000/60base pairs/sec=33 base pairs/sec

Comments: Article also gives info on distance between origins of replication: For a human cell, the approximately 3,000,000 kb genome is replicated in about 8 h. Since the rate at which replication forks plow through chromatin is no greater than~ 2 kb/min, bidirectional orgins would need to be spaced at roughly 2000 kb intervals to finish replication in the allotted time.

21. Rate of transcription by RNA polymerase \sim 70 bp/sec

70 nt/sec for E. coli (BNID 100060)

Ref: (1) Dennis, P. P., and H. Bremer. 1974. Differential rate of ribosomal protein synthesis in Escherichia coli B/r. J. Mol. Biol. 84:407–422.

PMID 4618855 (2) Dennis PP, Nomura M. Stringent control of ribosomal protein gene expression in coli B/r Proc Natl Acad Sci U S A. 1974 Oct;71(10):3819-23. PMID 4610562

Comments:~80 nt/sec according to Benno Muller-hill, 1996, The Lac Operon:

A Short History Of A Genetic Paradigm, Publisher: Walter de Gruyter. Berlin. New York. Part 3.2 pp.134

~71.6 nt/sec mammalian tissue culture cell (BNID 100662)

Ref: Darzacq X. et al, In vivo dynamics of RNA polymerase II transcription, Nature Structural & Molecular Biology - 14, 796 - 806 (2007) PMID <u>17676063</u>

Measurement Method: parameter optimization of FRAP and photoactivation data in U2OS 200 copy gene array. LacI gene integrated into Human U2OS osteosarcoma cells **Comments**: See also average polymerase velocity and pause residence time. YFP-Pol II is amanitin resistant allele.

22. Rate of translation by the ribosome ~20 aa/sec 12-21 aa/sec for *E. coli* (BNID 100059)

Oct71(10):3819-23 PMID 4610562

Ref: Bremer, H., Dennis, P. P. (1996) Modulation of chemical composition and other parameters of the cell by growth rate. Neidhardt, et al. eds. Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology, 2nd ed. chapter 97, pp. 1559, Table 3

Primary Source: (1) Young, R. and H. Bremer (1976). "Polypeptide-chain-elongation rate in Escherichia coli B/r as a function of growth rate." Biochem J 160 PMID 795428 (2): 185-94

Dennis, P. P., and H. Bremer. Differential rate of ribosomal protein synthesis in Escherichia coli B/r. J Mol Biol. 1974 Apr 1584 PMID 4618855 (3):407-22. Dennis, P. P., and M. Nomura. 1974. Stringent control of ribosomal protein gene expression in e.coli, PNAS 1974

Comments: lower value is for slow division rate (100 minutes) and higher value is for fast division rate (24 minutes)

Genomes, Mutation and error rates

Genome size:

E. $coli \sim 4 \times 10^6$ bp (BNID 100269)

Ref: Blattner FR et al, The complete genome sequence of Escherichia coli K-12, Science. 1997 Sep 5277(5331):1453-74. PMID <u>9278503</u>

Comments: The 4,639,221-base pair sequence of Escherichia coli K-12 is presented.

S. cerevisiae \sim 12×10⁶ bp (BNID 100459)

Ref: http://www.yeastgenome.org/cache/genomeSnapshot.html

Comments: The Budding yeast genome size is 12,156,676 base

Pairs as of May 6th 2009

C. elegans $\sim 100 \times 10^6$ bp (BNID 101363)

Ref: Hillier LW, Coulson A, Murray JI, Bao Z, Sulston JE, Waterston RH. Genomics in C. elegans: so many genes, such a little worm. Genome Res. 2005 Dec15(12):1651-60 PMID

Comments: The complete Caenorhabditis elegans genome sequence, fully contiguous telomere to telomere totals 100,291,840 bp.

D. melanogaster $\sim 120 \times 10^6$ bp (BNID 100199)

Ref: Adams MD et al, The genome sequence of Drosophila melanogaster. Science. 2000 Mar 24287(5461):2185-95. PMID 10731132

Arabidopsis $\sim 157 \times 10^6$ bp (BNID 104000)

Ref: Bennett MD, Leitch IJ, Price HJ, Johnston JS. Comparisons with Caenorhabditis (approximately 100 Mb) and Drosophila (approximately 175 Mb) using flow cytometry show genome size in Arabidopsis to be approximately 157 Mb and thus approximately 25% larger than the Arabidopsis genome initiative estimate of approximately 125 Mb Ann Bot (Lond). 2003 Apr91(5):547-57. PMID 12646499

Mouse $\sim 3 \times 10^9$ bp (BNID 100305)

Ref:http://wormlab.caltech.edu/briggsae/genomeSize.html

Human $\sim 3 \times 10^9$ bp (BNID 100396)

Ref: International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature. 2004 Oct 21431(7011):931-45. PMID 15496913 Comments: Value refers to Euchromatic genome. For heterochromatic genome see BNID 101484

Mutation rate in DNA replication $\sim 10^{-9}$ per bp in *E. coli* 24.

5.4E-10 mutation/bp/replication (BNID 100263)

Ref: Drake JW, A constant rate of spontaneous mutation in DNA-based microbes.Proc Natl Acad Sci U S A. 1991 Aug 1588(16):7160-4 Table 1 PMID 1831267

Measurement Method: Value is mean of two strains-hisGDCBHAFE (one measurement) and LacI (2 measurements)

Mutation rate in DNA replication 5×10⁻¹¹ per base pair per replication in human (BNID 100414) Ref: Drake JW, Charlesworth B, Charlesworth D, Crow JF. Rates of spontaneous mutation. Genetics. 1998 Apr148(4):1667-86 PMID 9560386

25. Error rate in transcription $\sim 10^{-4}$ per nucleotide (BNID 103453)

> Ref: Hani S. Zaher and Rachel Green, Fidelity at the Molecular Level: Lessons from Protein Synthesis. Cell Volume 136, Issue 4, 20 February 2009, Pages 746-76 PMID 19239893 **Primary Source**: Bouadloun F, Donner D, Kurland CG., Codon-specific missense errors in vivo, EMBO J. 2 (1983), pp.1351–1356. PMID <u>10872330</u> AND Rosenberger RF, Foskett G. An estimate of the frequency of in vivo transcriptional errors at a nonsense codon in Escherichia coli. Mol Gen Genet. 1981 183(3):561-3 PMID 7038382

Comments: This level of accuracy is lower than that of DNA replication and similar to

that of translation.

Error rate in translation $\sim 10^{-3}$ - 10^{-4} per amino-acid (BNID 103454)

Ref: Hani S. Zaher and Rachel Green, Fidelity at the Molecular Level: Lessons from Protein Synthesis. Cell Volume 136, Issue 4, 20 February 2009, Pages 746-76 PMID 19239893 **Primary Source**: (1) Bouadloun, D. Donner and C.G.

Kurland, Codon-specific missense errors in vivo, EMBO J. 2 (1983), pp. 1351–1356. PMID <u>10872330</u> AND (2) P. Edelmann and J. Gallant, Mistranslation in E. coli, Cell 10 (1977), pp. 131–137. PMID <u>138485</u>

Comments: This level of accuracy is lower than that of DNA replication and similar to that of transcription