Biothermodynamics, Chances and Problems

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1.Biothermodynamics
Overview, Historical Remarks

2.Structure of Thermodynamics

3.Biomolecules and Biofluids
DMPC-EOS (E2)

4.Proteins
Denaturation (E3), Adsorption (E4), Aggregation

5.Metabolism of Bacteria
Allometry, Thermal Limits of Life

6.Biocalorimetry
Medical Application (E6)

7.Bioreactors
Fermentation of Wine (E6)
Sterilization Process (E7)

8.Downstream – Processing

Literature

Biothermodynamics (BTH):

Application of Thermodynamics, i.e.Thermostatics (TST) and Thermodynamics of Irreversible Processes (TIP) to biological and bioengineering Systems.

Biotechnology (BT): Technology using living systems like cells, bacteria, fungi etc. as chemical reactors.

White BT	Industrial sized biocatalytic processes (fermentation) Breweries, Production vitamine B12, steroid hormones etc.;
Green BT	Plants and transgene variations for production of biofuels etc. in biorefineries;
Red BT	Medical applications of substances and processes related to living organisms, as for example interferones etc. (cancer,viruses)
Yellow BT	Pharmaceutical molecules, recombinant proteins, penicilline and other fungi;
Blue BT	Seawater based microorganisms as reactors; extremophiles... Extraction noble metals from seawater, production of new molecules

Fields of Research in Biothermodynamics

2nd Int. Symposium on Biothermodynamics
DECHEMA, Frankfurt am Main, February 21-22, 2008

Biomolecules

 # Protein adsorption on surfaces

 # Protein folding, interactions and stability

Bacteria

 # Active masstransport in biological membranes

 # Thermodynamics of metabolic pathways

 # Intracellular Thermodynamics

Bioreactors

 # Biocalorimetry

 # Thermodynamics of downstream processing

 # Thermodynamics in bio-logical energy conver-sion processes

 # Thermodynamic aspects of Systems Bilogy

2.Basic Concepts of Thermodynamics*

Thermodynamic System (W. Schottky, 1929)
Boundaries, Set of Operations

Level of Description (Beschreibungsebene)

Set of state variables (external, internal),
Set of exchange processes and dynamic equations,
Set of equations of state

1st Law of Thermodynamics and concept of Energy
Conservation of energy and mass (E.Noether, ca. 1930)

2nd Law of Thermodynamics and concept of Entropy
Law of large numbers,Central limit theorem (van Kampen, J.Meixner,1960-)

3rd Law of Thermodynamics
(W. Nernst, M. Planck, ca. 1910)

*Thermodynamics: Phenomenological theory of many – particle - systems.

Thermodynamic System (W. Schottky, 1929)

Σ: Set of bodies surrounded by well defined boundaries exchanging with its environment (Σ^*) by external operations transfer energies as

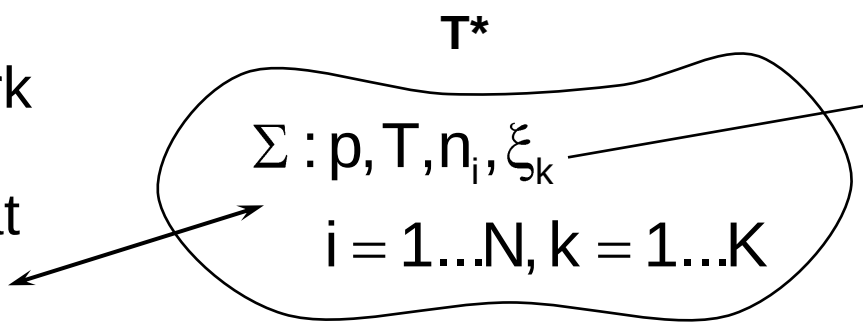
Work

Heat

Mass

.....

Information
(Living Systems)



Internal processes/variables
Mesoscopic Thermodynamics
Bridgeman, Flory, Kestin, Meixner, Muschik

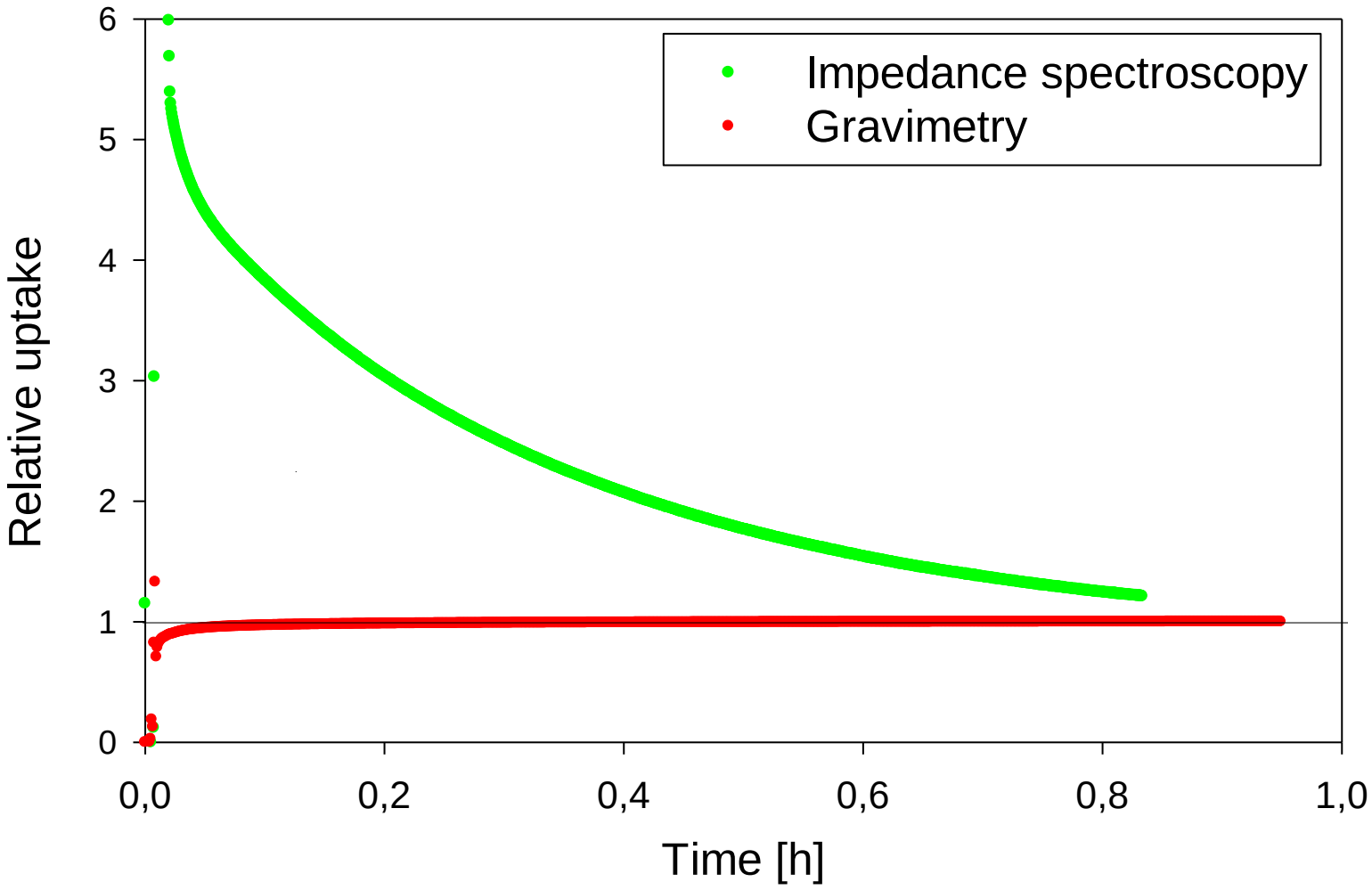
$$\Sigma^* : p^*, T^*, h^{(\alpha)}, s^{(\alpha)}, \mu_i^{(\alpha)}$$

$$\alpha = 1...A$$

External & Internal Processes: Level of macroscopic description or state of system (Σ).

Internal Variables of Thermodynamic Systems, Examples

1. Glass: Transition Processes: amorphous phase $\rightarrow$ crystalline phase
2. Polymeric materials: Molecular relaxation processes
3. Gases & Liquids: Slow dissociation / recombination processes (radioactive decay) (H_2S/AC)
4. Liquid crystals: Phase transition processes
5. Dielectric-/Diamagnetic relaxation processes
6. Proteins in (ionic) solution: Structural- / Molecular-relaxation (denaturation- i.e. folding, unfolding processes)



Uptake curves of H_2S on MS 13X, T=298K

Gibbs Equation for $G = G(T, p, n_1 \ldots n_N, \xi_1 \ldots \xi_k)$

$$dG = -SdT + Vdp + \sum_{i=1}^{N} \mu_i dn_i - \sum_{k=1}^{K} A_k d\xi_k$$

Chemical reactions ($Q \leqq N - E$)

$$C_i = \sum_{e=1}^{E} \alpha_{ie} E_e \,, \qquad \sum_{i=1}^{N} \nu_{iq} C_i = 0 \,, \qquad q = 1 \ldots Q$$

Conservation of atomic numbers:

$$\sum_{i=1}^{N} \nu_{iq} \alpha_{ie} = 0 \,, \qquad e = 1 \ldots E \,, \qquad q = 1 \ldots Q$$

Chemical production of component (i):

$$n_i^c = n_i^* + \sum_{q=1}^{Q} \nu_{iq} (\gamma_q - \gamma_q^*) \,, \qquad i = 1 \ldots N$$

Gibbs Equation (T = const, p = const)

$$dG = -\sum_{q=1}^{Q} A_q^c d\gamma_q - \sum_{k=1}^{K} A_k d\xi_k$$

$$A_q^c = -\sum_{i=1}^{N} \mu_i \nu_{iq}$$

a) Restricted or frozen equilibria: $\xi_1 ... \xi_k$ = const … arbitrary value

$$A_q^c(T,p,\gamma_1 ... \gamma_Q, \xi_1 ... \xi_k) = 0 , \quad q = 1...Q$$

Reaction numbers $\rightarrow$ $\gamma_{qE} = \gamma_q(T,p,\xi_1 ... \xi_k)$

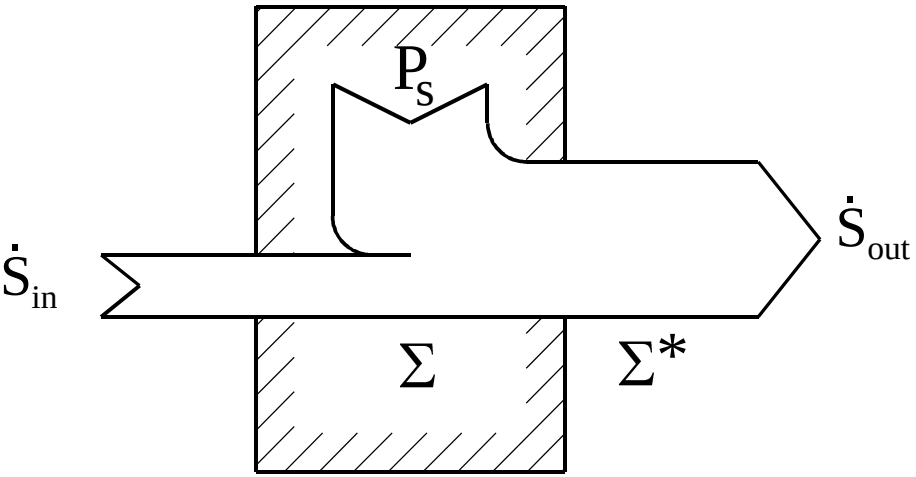
b) Full or unrestricted equilibria:

$$A_q^c(T,p,\gamma_1 ... \gamma_Q, \xi_1 ... \xi_K) = 0 , \quad q = 1...Q$$

$$A_k(T,p,\gamma_1 ... \gamma_Q, \xi_1 ... \xi_K) = 0 , \quad k = 1...K$$

$$\rightarrow \quad \gamma_{qE} = \gamma_q(T,p) , \ \xi_{kE} ... \xi_k (T,p)$$

Thermodynamics of Photosynthesis (E1)



Evaporation of Additional Water:

$$\dot{S}_{in} = \dot{S}_{out} + 0.24\,\frac{kJ}{mol\,K}\cdot\dot{n}_{GL}$$

$$2.2\,|\;\dot{S}_{H_2O_L} = \dot{S}_{H_2O_V} - 0.11\,\frac{kJ}{mol\,K}\cdot\dot{n}_W$$

$$\dot{n}_W = 2.2\cdot\dot{n}_{GL}$$

E. Schrödinger (~1940)

$$\underbrace{6\,CO_2 + 6\,H_2O}_{\dot{S}_{in}} \rightarrow \underbrace{C_6H_{12}O_6 + 6\,O_2}_{\dot{S}_{out} + 0.24\,\frac{kJ}{mol\,K}\cdot\dot{n}_{GL}}$$

2nd Law: $\dot{S}_{in} \leq \dot{S}_{out}$?

$$\left\| \begin{array}{l} 6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2 \\ +\,2.2\;H_2O_L \rightarrow 2.2\;H_2O_V \end{array} \right\|$$

3. Biomolecules and Biofluids

Biomolecules (proteins,enzymes etc.,aggregates of amino acids (MBM)
Spatial structure ...Stereochemistry,
Surface: polar & non-polar regions, electric charges.

Solvent molecules (water,alcohols,organc solvents etc.) (Mw <<< MBM)
polar & non-polar fluids, salts (ions)

Solvent molecule near surface of biomolecule is different from solvent molecule in the bulk phase.

Problems: Biomolecules as „subsystems“ of biofluids ?
Surface of biomolecule as sorbent for solvent particles ?

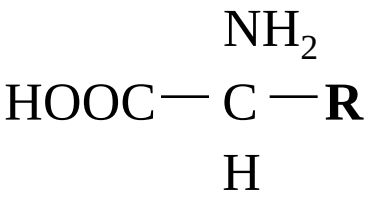
„State of biomolecules“ (native,denatured, etc.) ?

Interactions between biomolecules

Thermodynamics: **Internal Variables** of a system....**internal equations of state**

Amino Acids (AA) Selection (1), Bohinski (1979), Voet&Voet (1996)

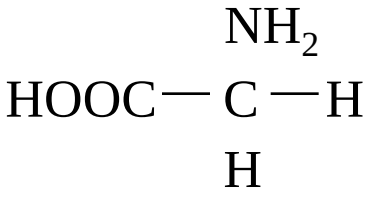
General Structure



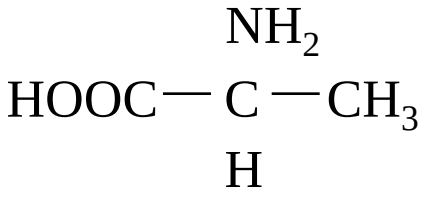
R-Group:
- Aliphatic
- Aromatic
- Hydroxyl
- Acidic
- Basic
- Imino
- Sulfur

Aliphatic AA

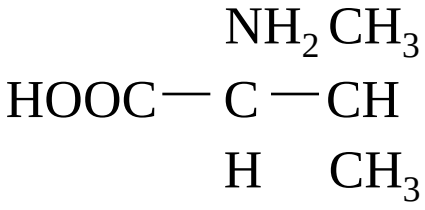
Glycine (gly)



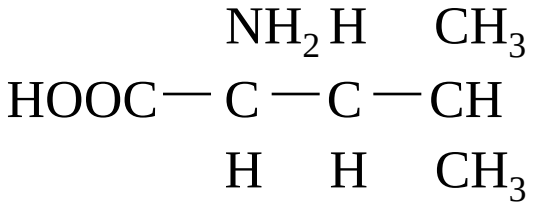
Alanine (ala)



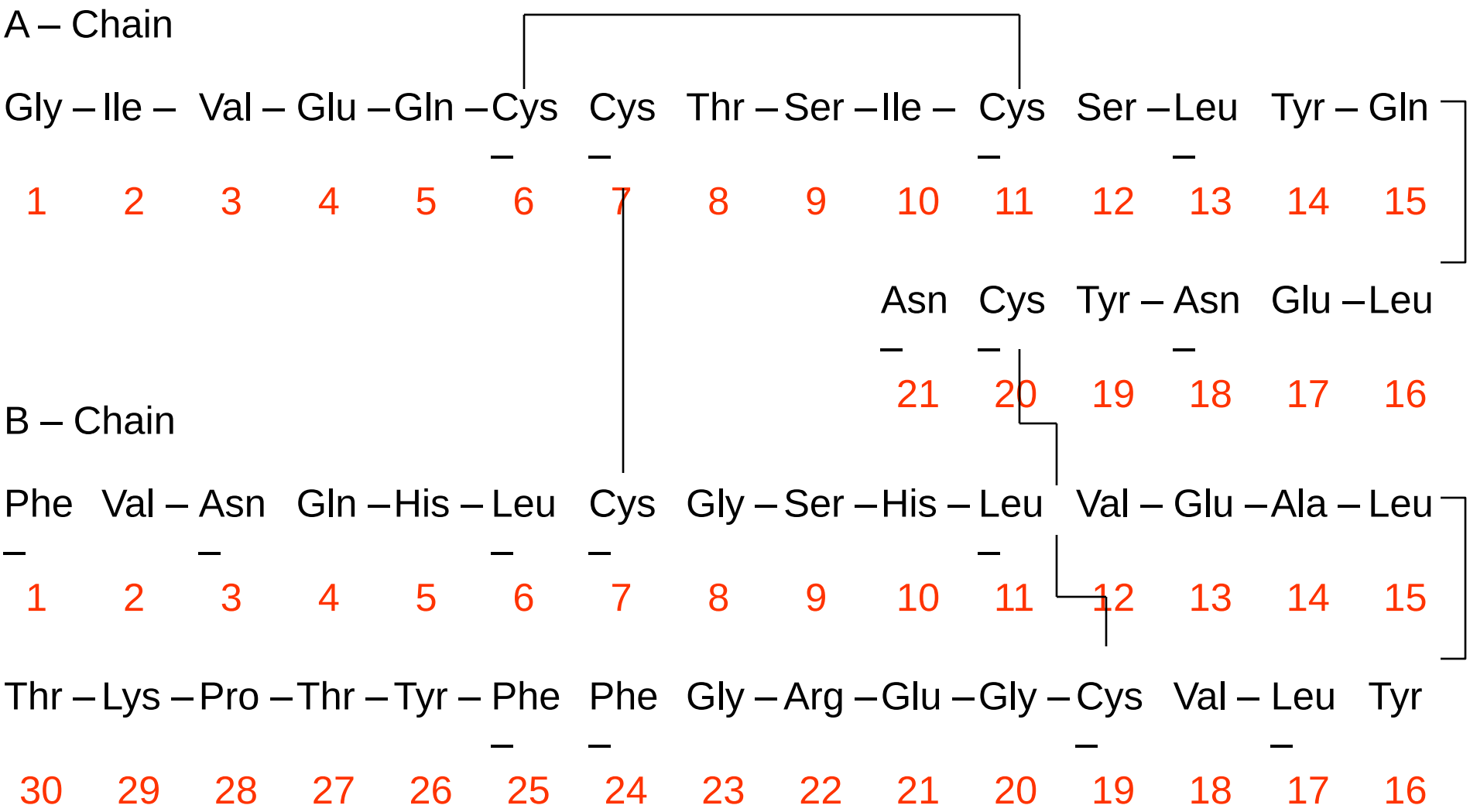
Valine (val)



Leucine (leu)

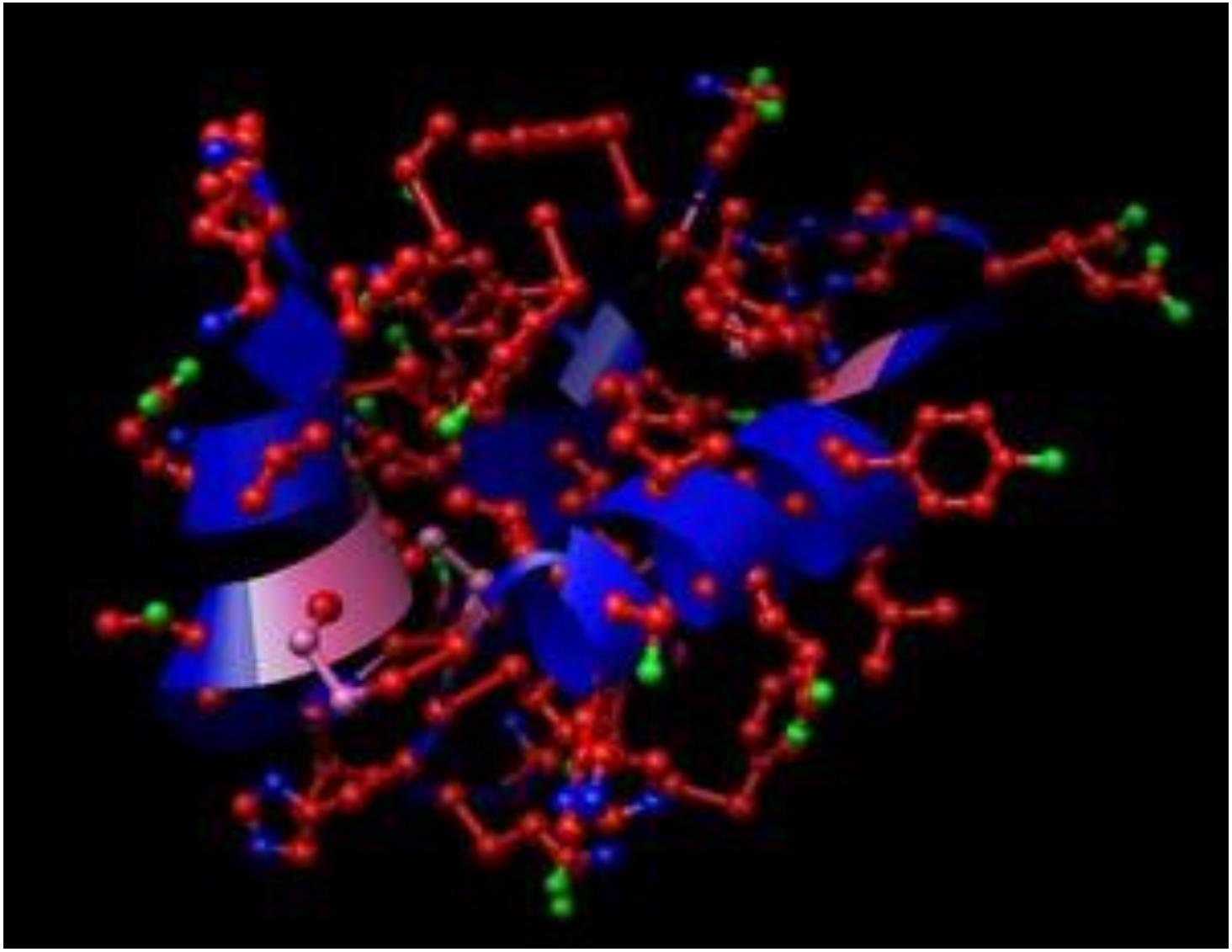


etc.



Primary Structure of Human Insulin (Roempp)

Polypeptide (A, B), M ≅ 6000 D



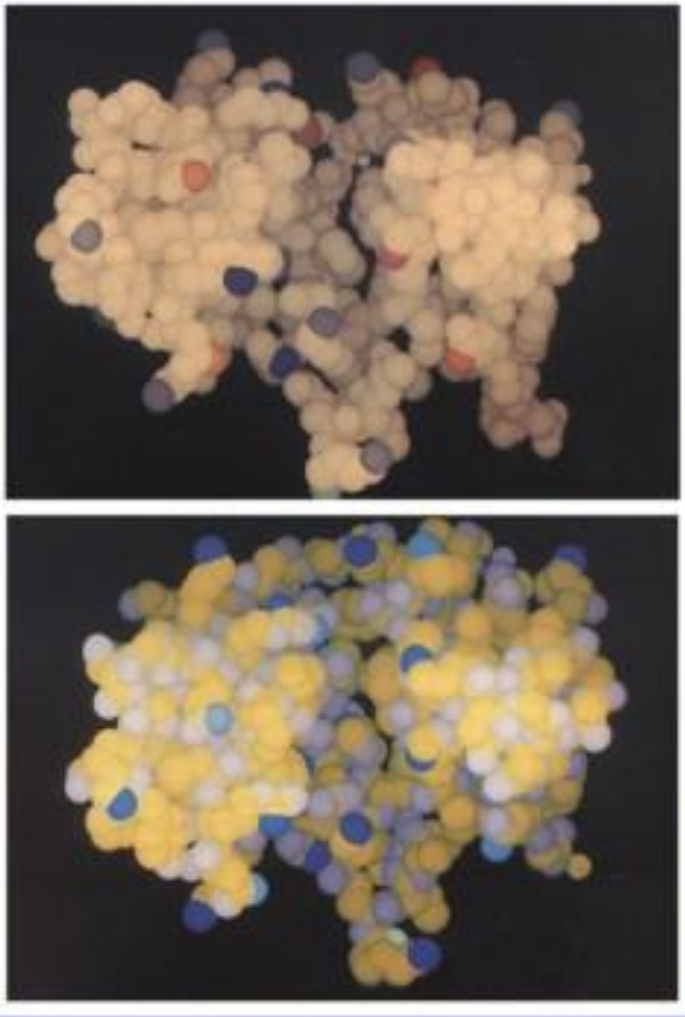
Insulin-Molecule, Source: Wikipedia 2005

proteins

computer graphics of lysozyme

Re: W. Norde,
Colloids and Interfaces in
Life Sciences, 2005

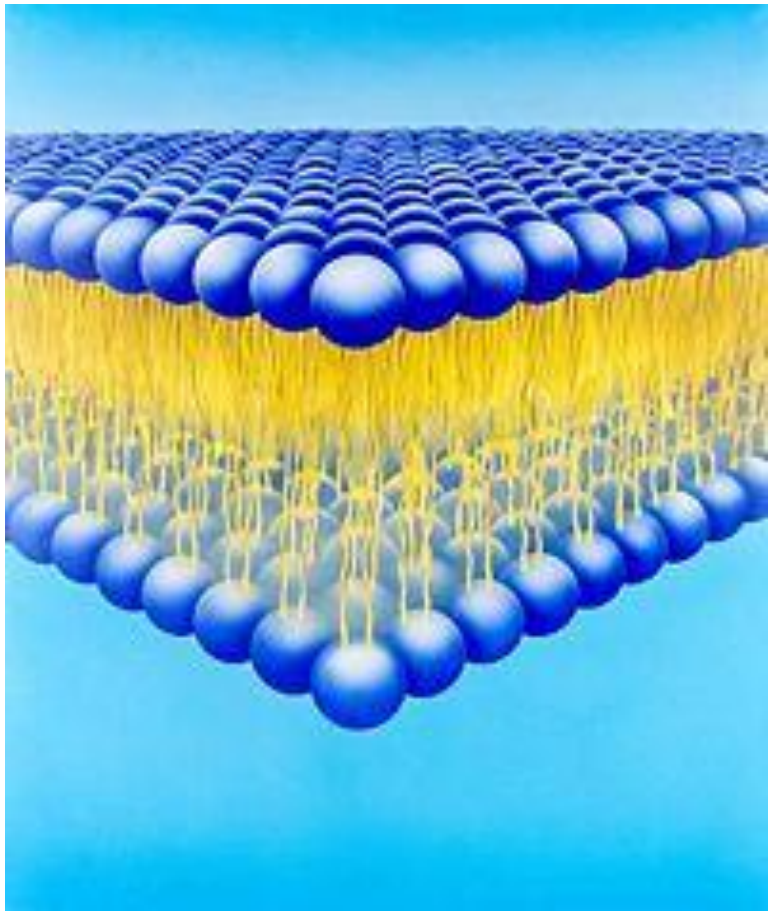
secondary structure



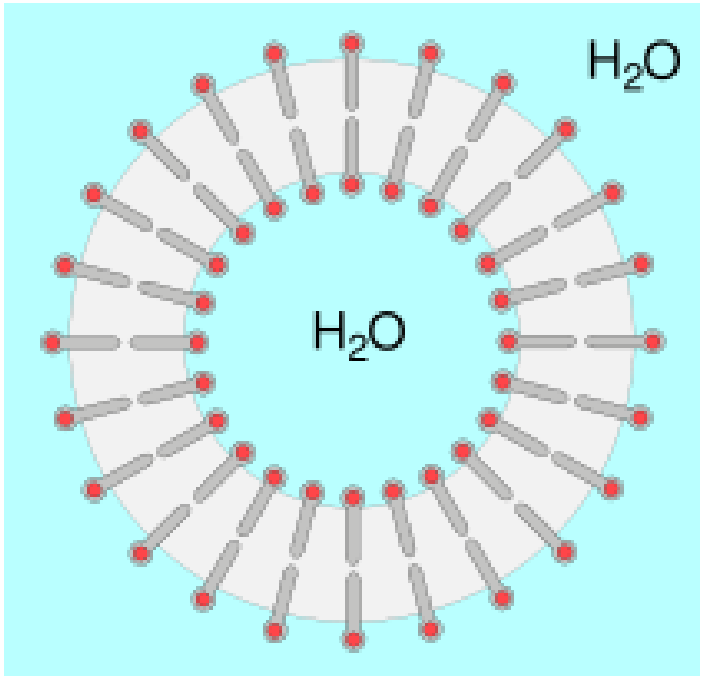
tertiary structure

Cell Membranes: Thermal Equation of State (E2)

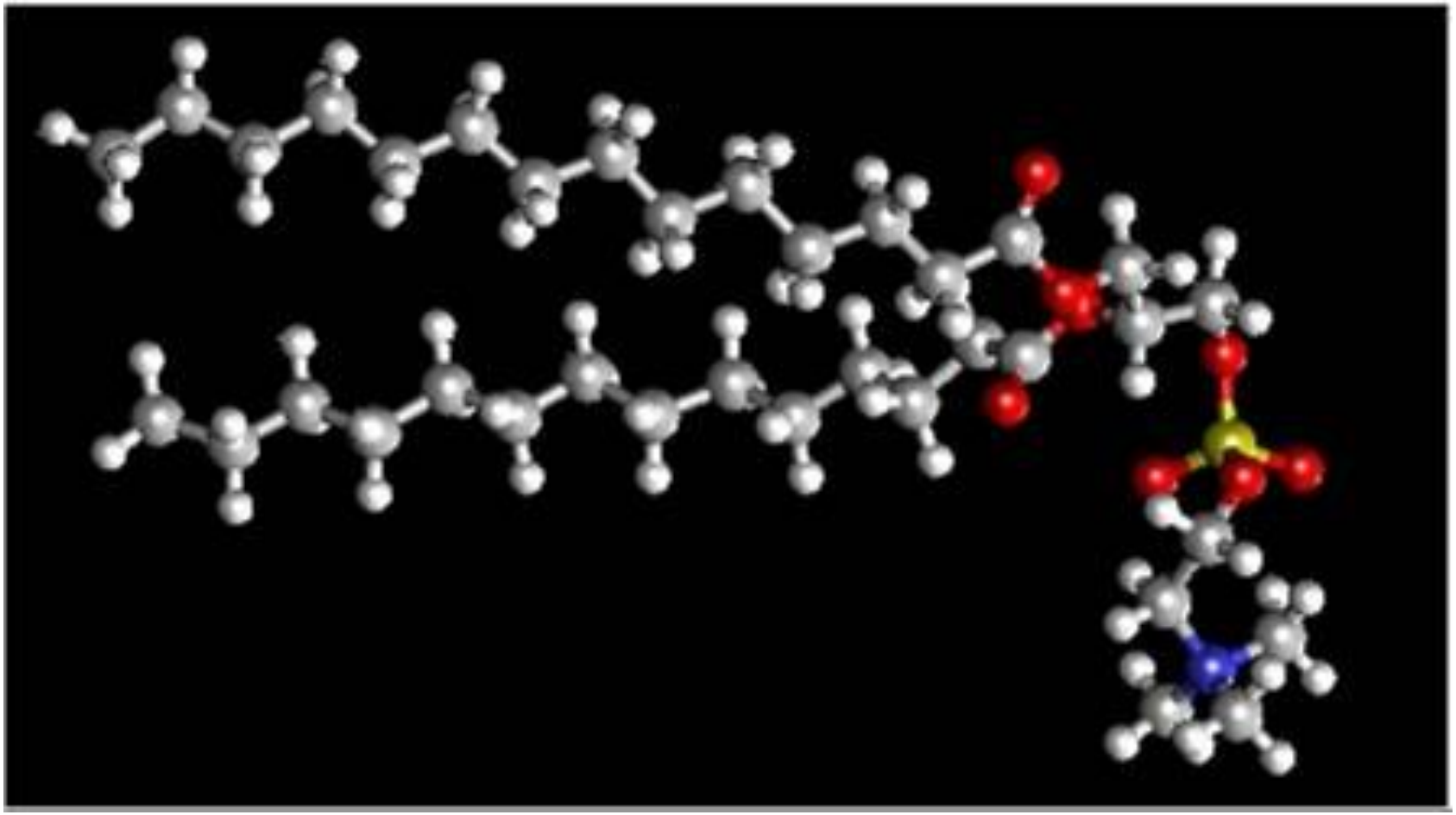
Double layer of lipid molecules
Polar „heads“ – Non-polar „tails“



Lipid bilayer forming a micelle

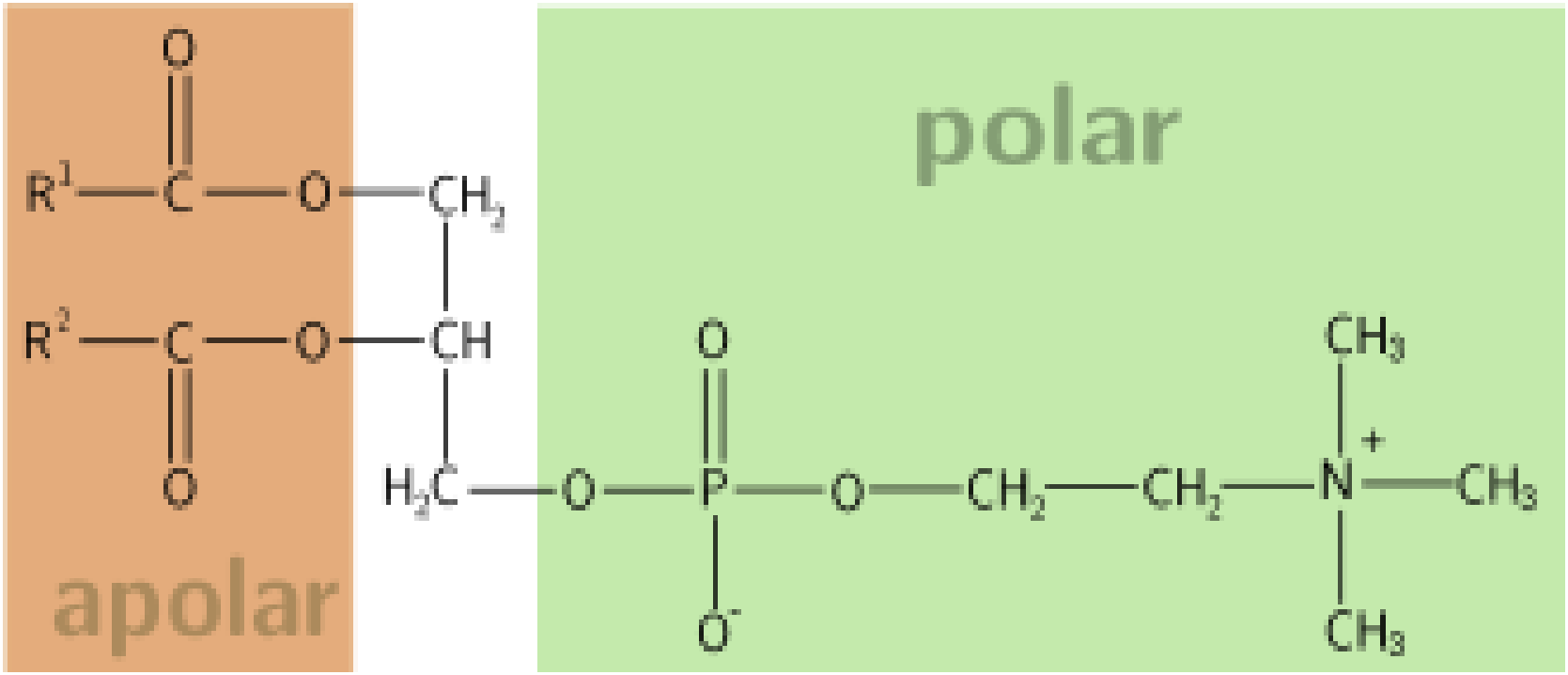


1,2-Dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC)



DMPC – Strukture: Phosphatidylcholine / Lecithine

Fatty acids

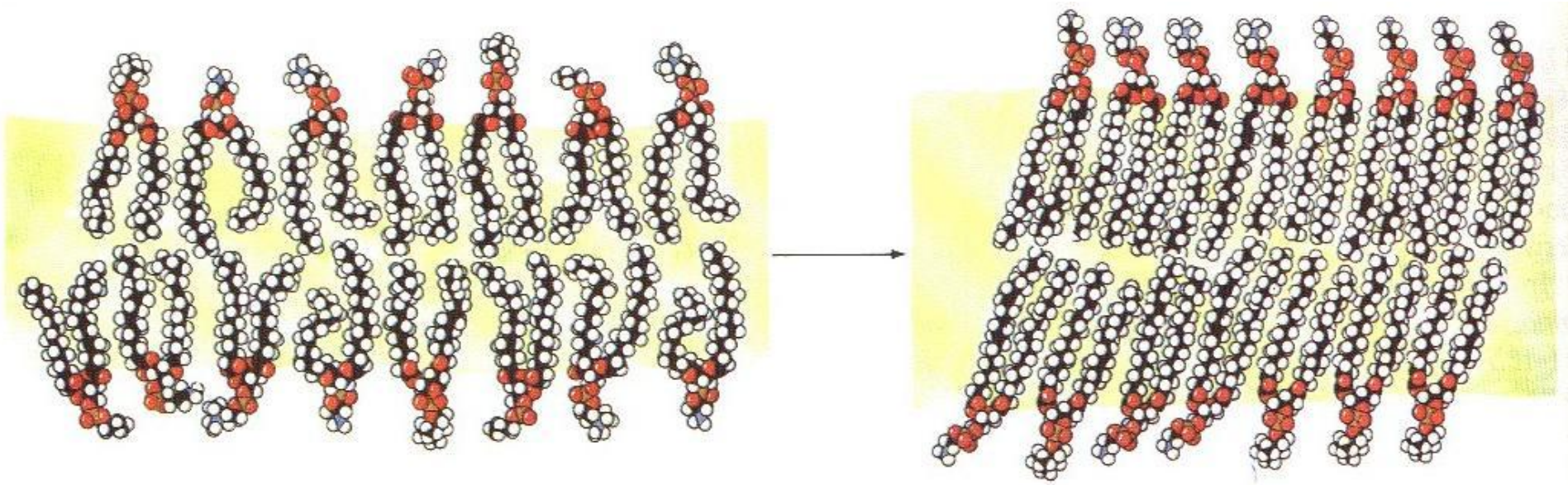


Glycerine CH2 – CH – CH2
/ / /
OH OH OH

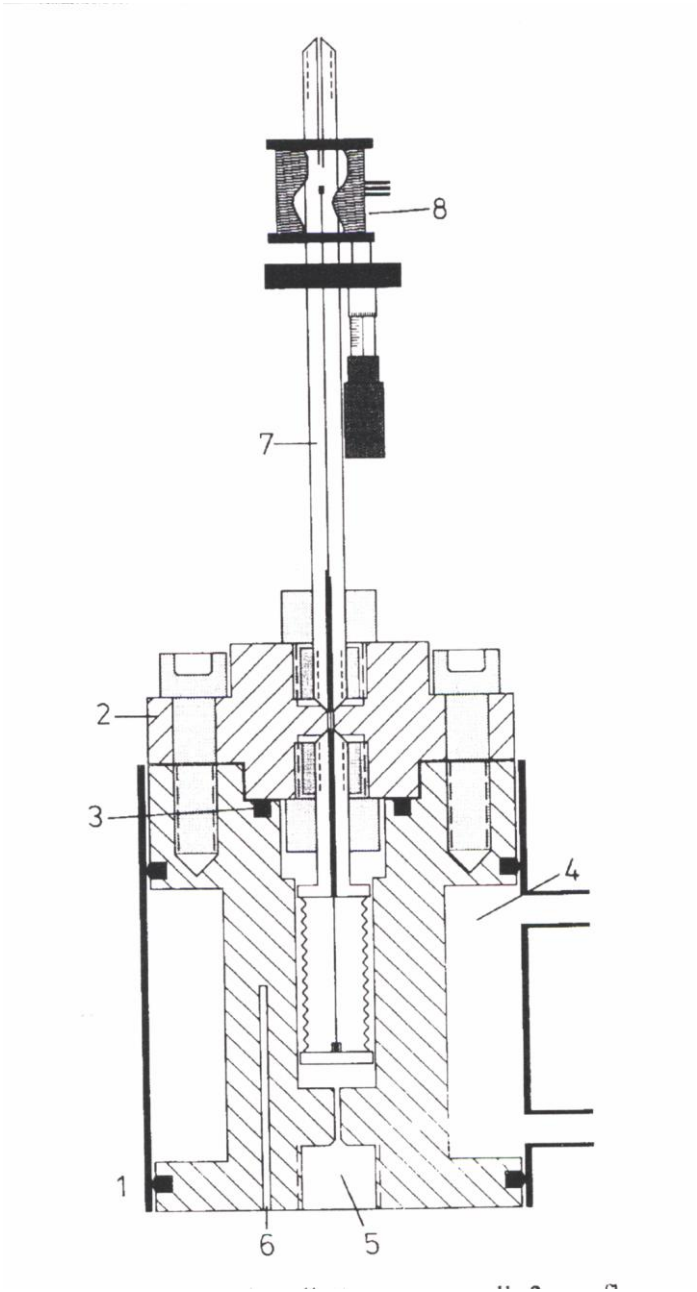
Choline

Lipid Membranes, Phase Transition Fluid - Gel

$T > T_t(p, ...)$ $T < T_t(p, ...)$



Lipid by layer formed of phosphatidylcholine (Voet&Voet, p. 288)



Volumetric Cell T = (0 – 100) C P < 250 MPa

1 Pressure cell

2 Top flange

3 Viton O-ring

4 Thermostate

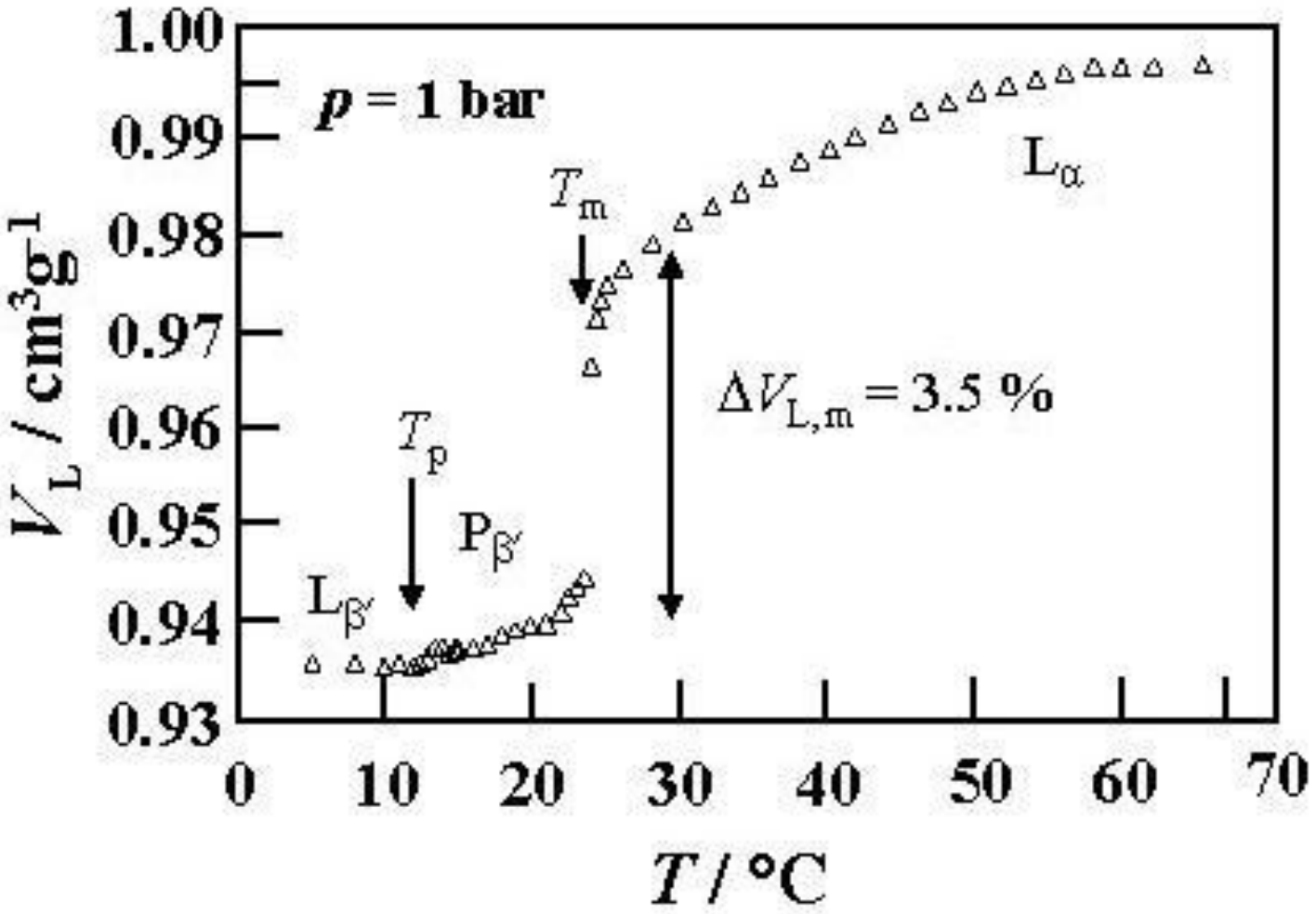
5 High pressure nut

6 Thermocouple inlet

7 High pressure pipe

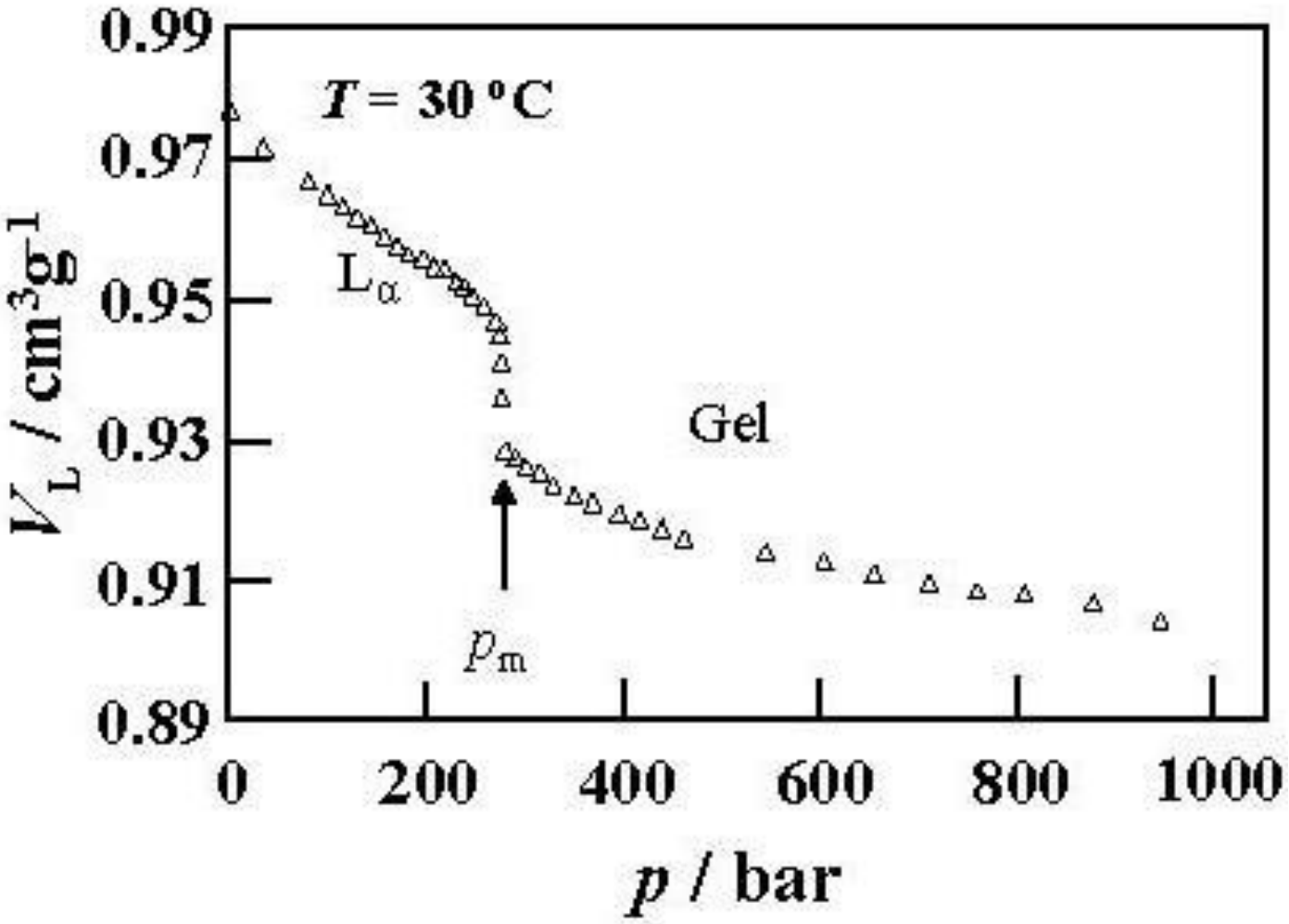
8 Inductive coil

Ref. Böttner M. et al., High Pressure Volumetric Measurements on Phospholipid Bilayers, Z. Physik.Chemie 184(1994),p.205



Temperature and pressure dependence of the specific volume of DMPC*) in water. (R. Winter, JNE 6-22, 2007)

*)1,2-dimyristoyl-s,n-glycero-3-phosphatidylcholine

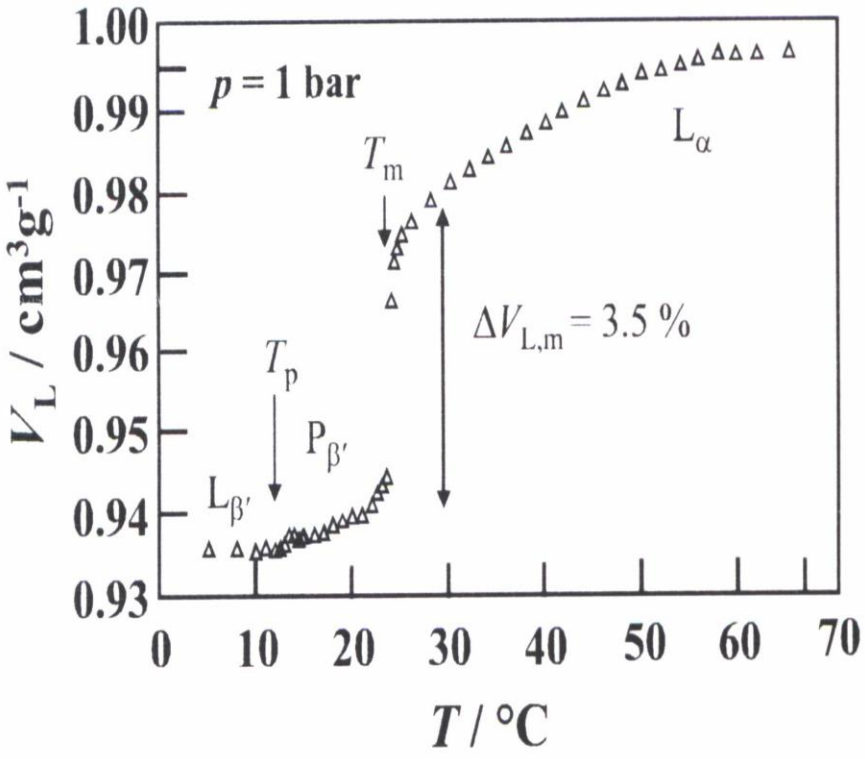


Temperature and pressure dependence of the specific volume of DMPC*) in water. (R. Winter, JNE 6-22, 2007)

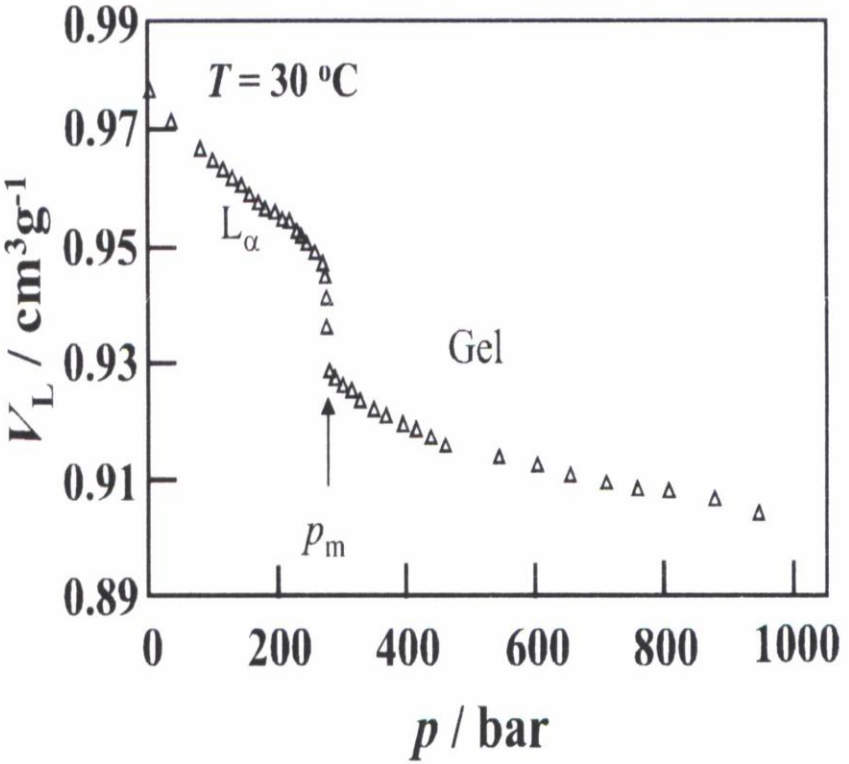
*)1,2-dimyristoyl-s,n-glycero-3-phosphatidylcholine

p,v,T- Data of DMPC Bylayers, Phase Transition Fluid-Gel

T,v-Data at p=1bar



p,v-Data at T=30 C



Measurement Method: High Pressure cell,volumetry.

Ref.: R.Winter etal.,JNE 32(1),2007,p.41

DMPC Thermal Equation of State (EOS)

Aliphatic tails of DMPC-molecules may aggregate/adsorb on each other.

Degree of aggregation:

$$\alpha(v) := \frac{v_{.0} - v}{v_{.0} - b_{.0}} \qquad 0 < \alpha(v) < 1$$

Fluid state Gel state

Free volume

$$\beta(v) := \frac{v - b_{.0}}{v_{.0} - b_{.0}}$$

EOS:

$$p(\alpha, T) := A(T)\cdot\alpha + B(T)\cdot\alpha^2 + D(T)\cdot\alpha^3 + C(T)\cdot\frac{\alpha^\gamma}{1-\alpha^\gamma} \qquad \gamma := 1$$

Fractality

Virial expansion ...

Adsorption term

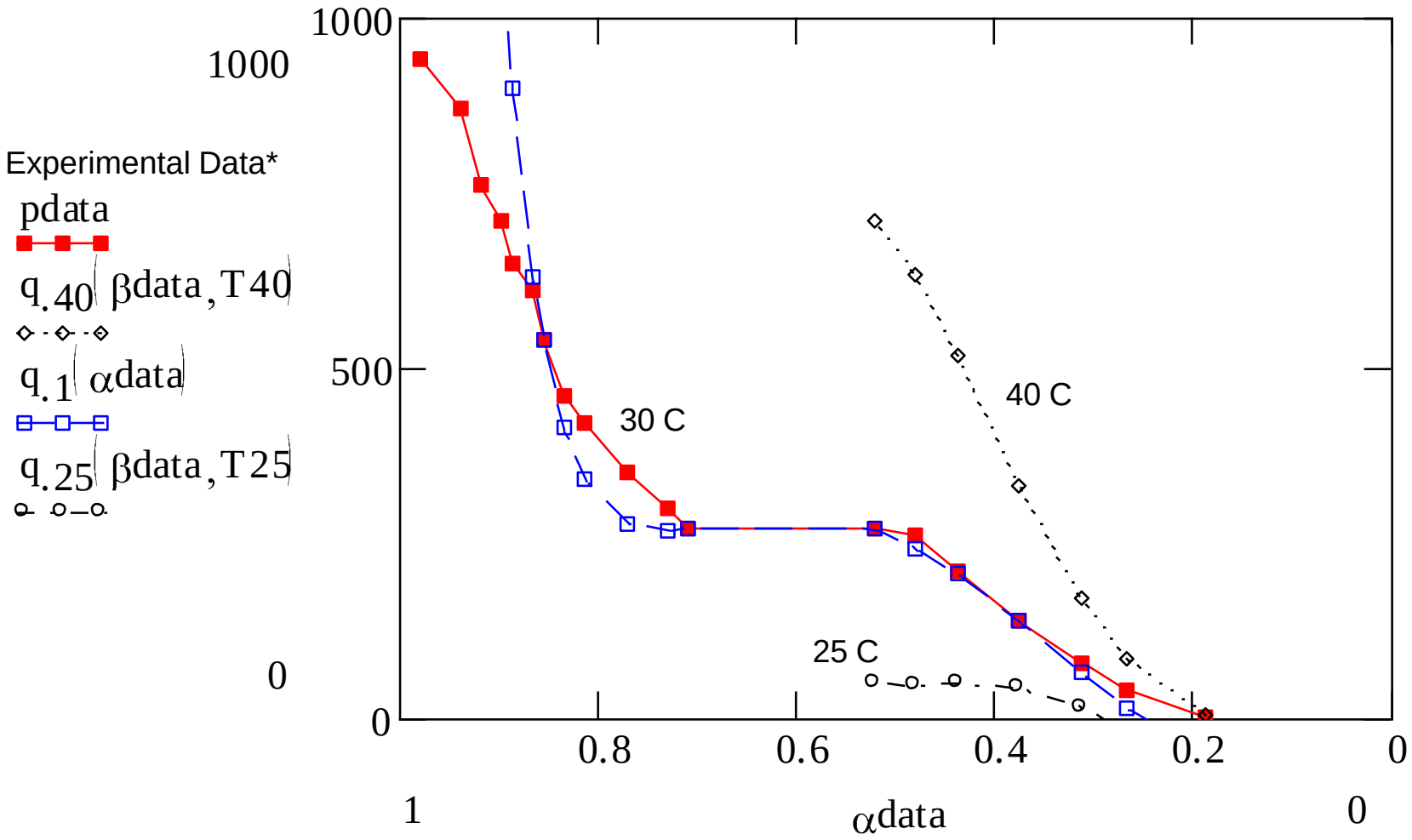
$$A(T) := A_{.0}\cdot\left[1 + a\cdot(T - T_{.0})\right]$$

.....

$$D(T) := D_{.0}\cdot\left[1 + d\cdot(T - T_{.0})\right]$$

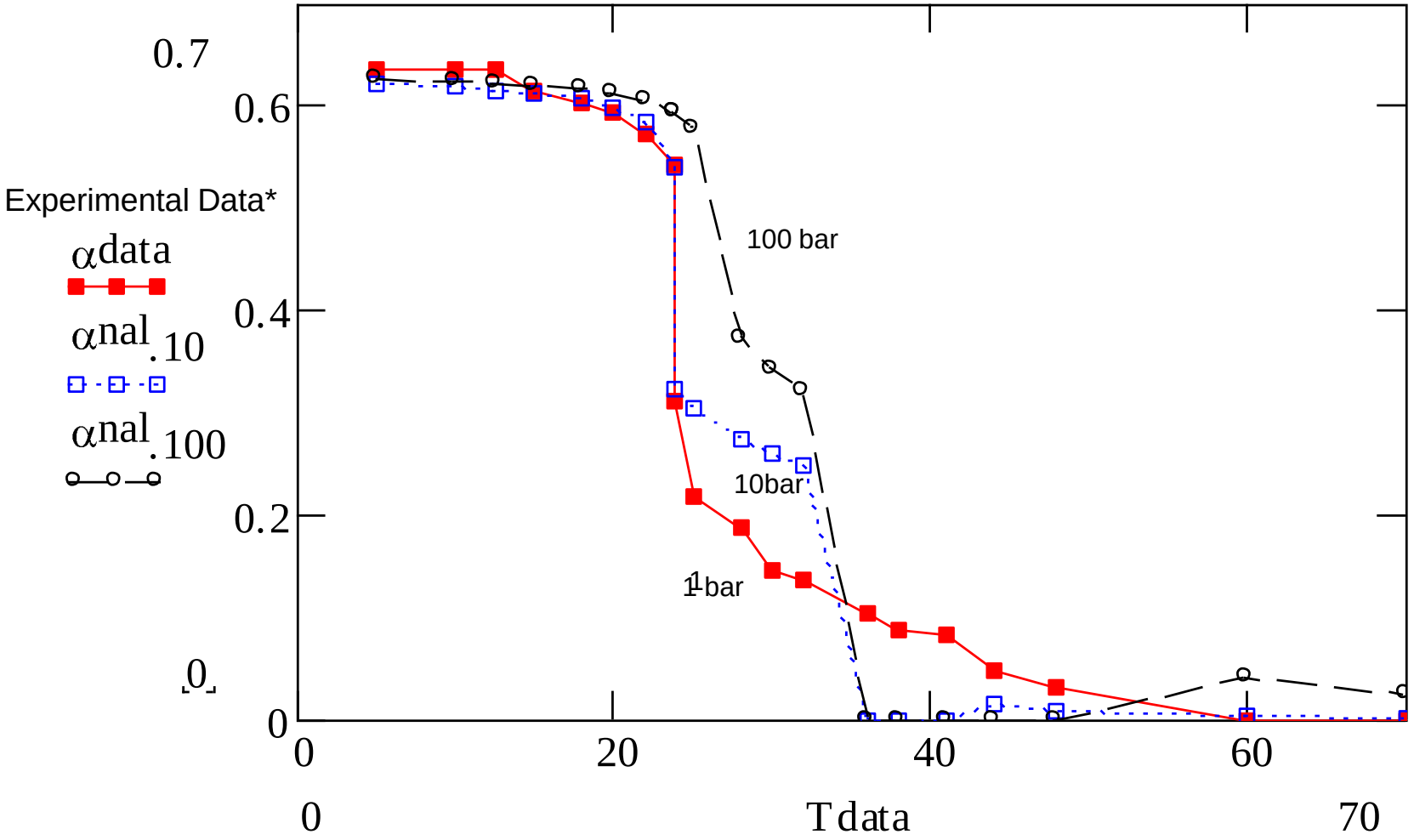
A= -1873 bar	a=-0.54
B=7942	b=-0.051
D=-8997	d=-0.429
C=333.34	c=-2.534

DMPC Thermal Equation of State (EOS)
Correlation of Isothermal Data



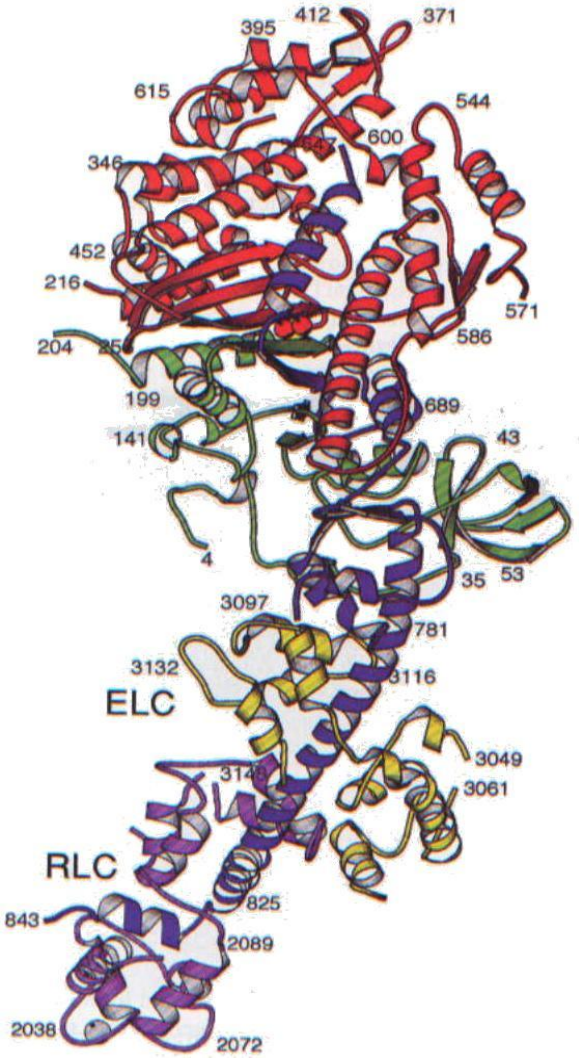
* R. Winter et al.,JNE 32(2007),p.41

DMPC Thermal Equation of State (EOS)
Correlation of Isobaric Data



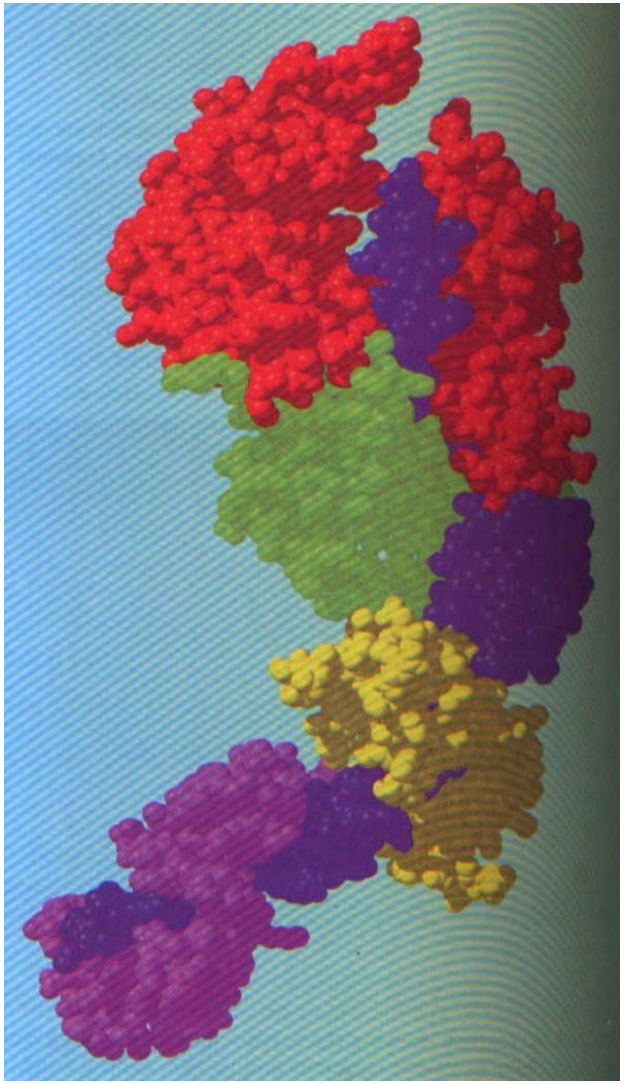
*R.Winter et al., JNE 32(2007),p.41-

4.Proteins (Example): Myosin from Chicken Muscle



Secondary Structure

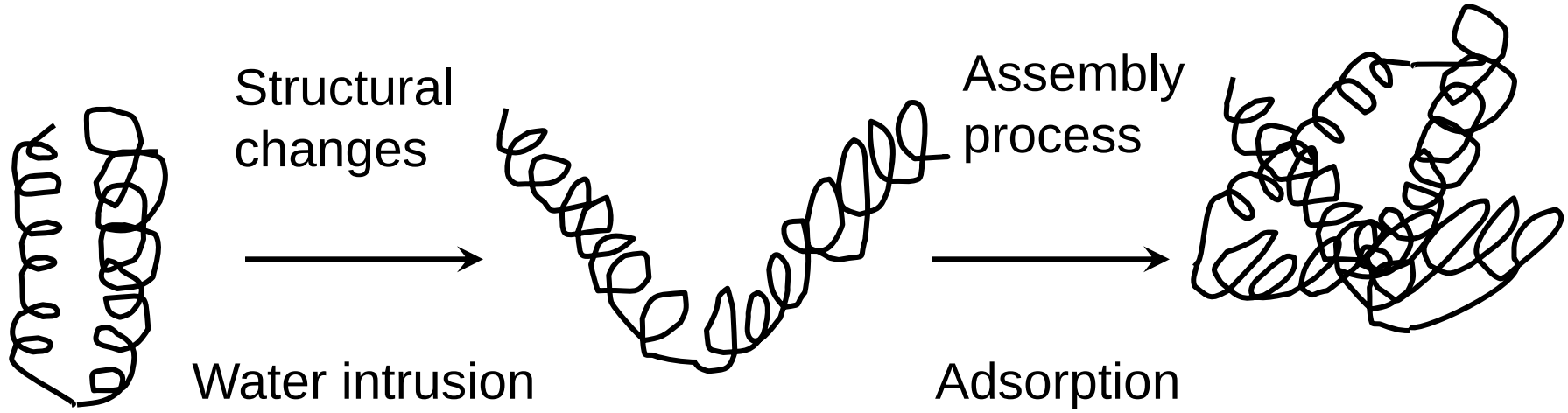
Voet&Voet
Biochemistry
Wiley,N.Y.
1995



Tertiary Structure (X-Ray)

Proteins: Unfolding and Aggregation (E3) (Alzheimer Disease)

→ Loss of bioactivity



Native Protein (N)
Dense packing

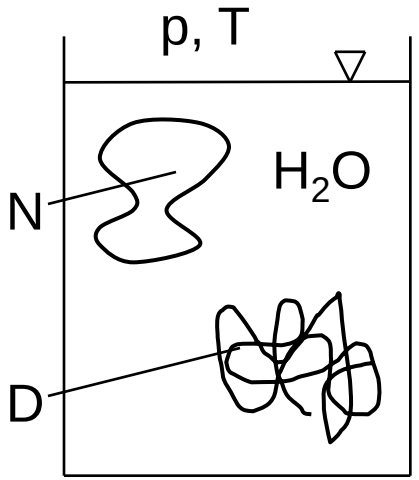
Stimulated Transition State, Defolding (D)

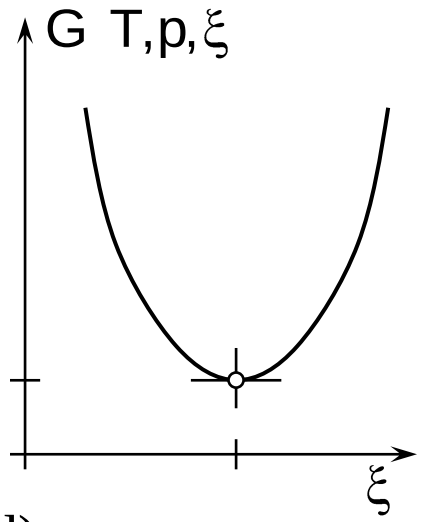
Non-native state
Aggregation (A)
Self-adsorption

$M \cong 20000\,D$

$\varepsilon_r \cong 10$

Denaturation of Proteins, Thermodynamic Analysis, Equilibria





N ... Native (folded) state

D ... Denaturated (unfolded) state

N↔D Quasichemical reaction (ξ)

$$G = G\ T,p,n_N,n_D = \mu_N n_N + \mu_D n_D \quad 1$$

$$dG = -S\,dT + V\,dp + \mu_N\,dn_N + \mu_D\,dn_D \quad 2$$

Equilib.: $G \rightarrow \text{Min}, T = \text{const},$

$$p = \text{const}, n = \text{const}$$

$$dG = 0\ ,\ d^2G > 0 \quad 3$$

Reaction parameter:

$$n_N = n_{N0} - \xi\ ,\ dn_N = -d\xi$$

$$n_D = n_{D0} + \xi\ ,\ dn_D = d\xi$$

$$2,3 : dG = -\mu_N + \mu_D\ d\xi = 0$$

$$\rightarrow \quad \underline{\mu_N = \mu_D} \quad 4$$

$$\mu_i = \mu_{i0}\ T,p + RT\ln\ \gamma_i x_i\ ,\ i = D,N \quad 5$$

$$5\ ,\ 4\ \underbrace{\mu_{N0} - \mu_{D0}} = RT\ln\ \gamma_D x_D / \gamma_N x_N$$

$$-\Delta G = RT\ln K_{eq}\ T,p$$

$$K_{eq} \doteq \frac{\gamma_D x_D}{\gamma_N x_N} = e^{-\Delta G/RT} \quad 6$$

Ideal solution: $\gamma_D = \gamma_N = 1$

Real solution: Calor.measurements

Thermal Denaturation of Myoglobin

153 Amino acids

Seize: (44x44x25)$Å^3$

Molecular Weight $\approx$ 18kD

N ... Native (folded) State

D ... Denaturated (unfolded) State

Equilibrium at T=const, p=const

$$\Delta G_{DN}(p,T) = -R\,T\,\ln\left(\frac{\gamma_D\,x_D}{\gamma_N\,x_N}\right)$$

$\Delta G_{DN} = \mu_{D0} - \mu_{N0}$

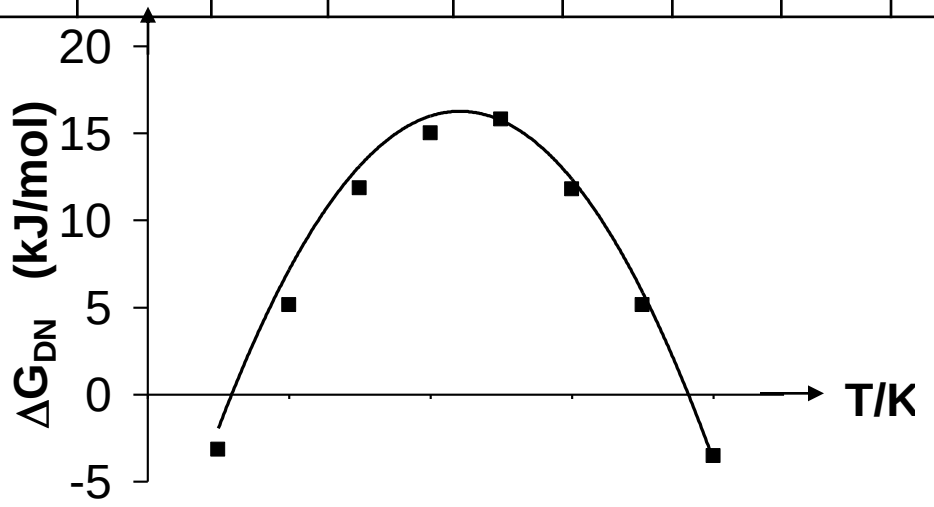
Approx.: $\gamma_D = \gamma_N = 1$

$\Delta G_{DN} > 0 \rightarrow x_D \ll x_N$...N...stable

$\Delta G_{DN} < 0 \rightarrow x_D \gg x_N$...N...unstable

Experimental Data

T/K	270	280	290	300	310	320	330	340
ΔG_{DN} (kJ/mol)	-3.16	5.13	11.8	15	15.8	11.8	5.13	-3.53
ΔH_{DN} (kJ/mol)								
ΔS_{DN} (kJ/mol K)								

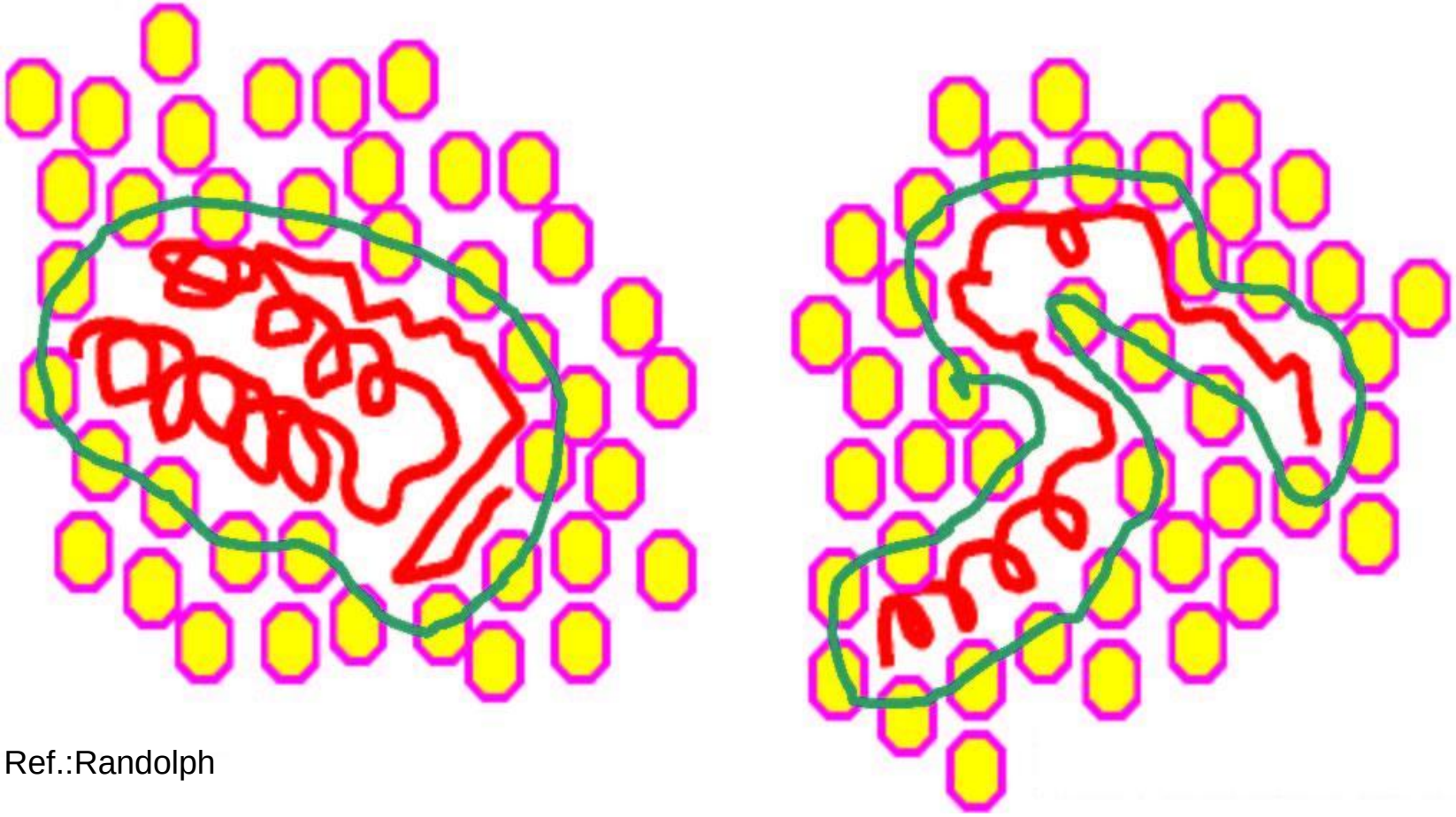


(U.von Stockar,EPFL,et al., MV Seminar,2000)

Protein(P) - Water(W) Interactions (E4)

P: Conformational Changes, Unfolding

W: Adsorption, Intrusion, Coating of (P): Stabilization



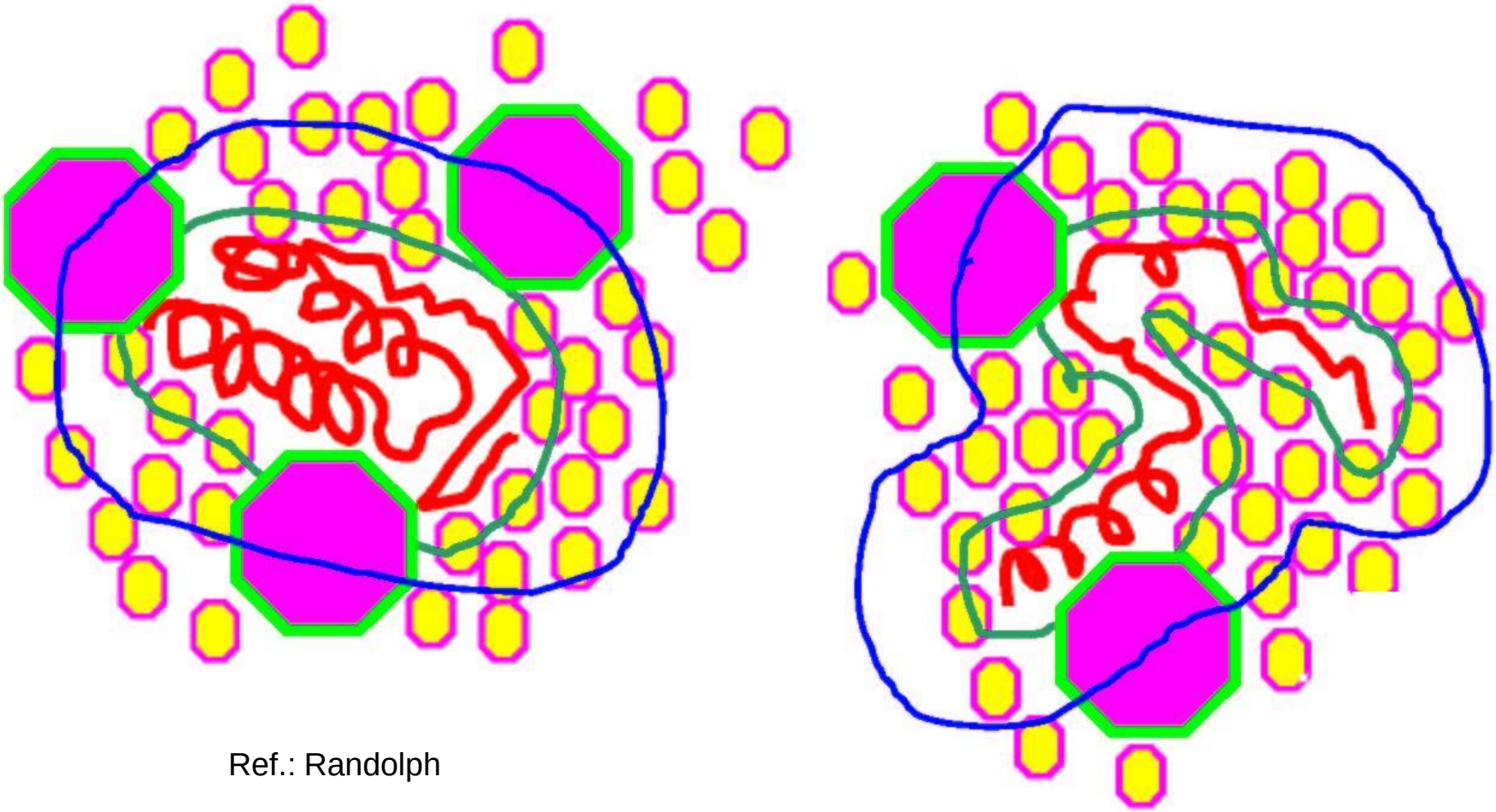
Ref.:Randolph

Native State (N):
compact, surface area small

Unfolded State (D):
expanded, surface area high

Protein(P) - Water(W) – Sugar(S) Interactions

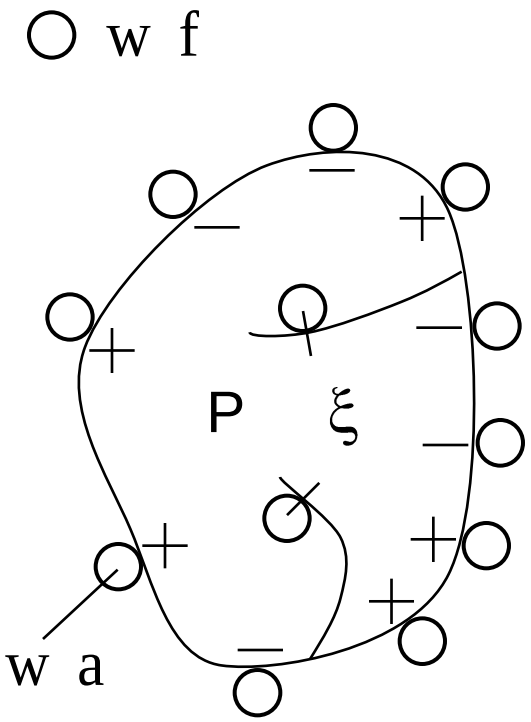
S: Adsorption, Desorption upon unfolding of protein.



Ref.: Randolph

SW: Coadsorption on surface may stabilize (P).

Hydratization Process of Proteins (E4)
Water Intrusion



Water:

$T, p, \mu_w^f = \mu_w^a = \mu$

Stimulus: Chemical potential of water: $\mu = \mu\ p, T, ...$

Response: Adsorption of water on P

AI: $n = n\ \mu, T = const\ = n_0 + H_0\ t\ \ \mu - \mu_0\ + O\ 2$

Number of Adsorption sites: ξ ... Internal variable!

a) $\xi = \xi_E = const$ ... equilibrium : $\xi = \xi_E\ (n, T = const)$

b) $\xi \neq \xi_E$ ... variable ... non-equilibrium:

$A = A\ (n, T = const, \xi) \neq 0$

Affinity: Measure for non-equ. deviation.

Hydratization Process of Proteins (Water Intrusion)

Thermostatics 1

Free energy of (P, w)-system:

$$F = F\ n, \xi, T\ = -S\,dT + \mu\,dn - A\,d\xi \quad , \quad T = \text{const}$$

$$\mu = \left(\frac{\partial F}{\partial n}\right)_{T,\xi} = \mu\ n, \xi, T\ \ldots \text{AI}$$

$$-A = \left(\frac{\partial F}{\partial \xi}\right)_{T,n} = -A\ n, \xi, T\ \ldots \text{IEOS}$$

External & internal or full equilibrium: $F \rightarrow \text{Min}$, $T = \text{const}$, $n = \text{const}$

$$A\ n, \xi, T\ = 0 \rightarrow \xi_E = \xi_E\ n, T\ = \text{const}$$

External equilibrium only (restricted equilibrium), $T = \text{const}$:

$A \neq 0$ ξ ... arbitrary value

Hydratization Process of Proteins (System: P, w(a))

Free Energy, Taylor Series

$$F\ n,\xi,T\ = F_{00} + F_{10}n + F_{01}\xi + \frac{1}{2!}\ F_{20}n^2 + 2F_{11}n\xi + F_{02}\xi^2\ + O\ 3$$

Thermodynamic Stability (2nd Law): $\left\|\partial^2 F/\partial n\,\partial\xi\right\| > 0\ ,\ F_{ik} = F_{ik}\ \ T$

$$\rightarrow F_{20} \geq 0\ ,\ \ F_{20}F_{02} - F_{11}^2 > 0\ ,\ \ F_{02} \geq 0$$

Reference State: $Z_0\ \ n_0, \mu_0, \xi_0, A_0 = 0, T$

Equations of State:

$$\mu = \ \partial F/\partial n\ _{\xi,T} \quad : \quad \mu - \mu_0 = F_{20}\ \ n - n_0\ + F_{11}\ \ \xi - \xi_0 \qquad 1$$

$$-A = \ \partial F/\partial \xi\ _{n,T} \quad : \quad -A = F_{11}\ \ n - n_0\ + F_{02}\ \ \xi - \xi_0 \qquad 2$$

Internal Equilibrium: $A\ \ n, \xi_E T\ = 0\ ,\ \xi_E - \xi_0 = -\frac{F_{11}}{F_{02}}\ \ n - n_0$

$$1 : \underline{\underline{n - n_0 = H\ \ \mu - \mu_0}}\ ,\ H = \frac{F_{02}}{F_{20}F_{02} - F_{11}^2} > H_0 = \frac{1}{F_{20}}$$

Hydratization Process of Proteins (System: P, w(a))

Thermodynamics of Processes

1st Law: $dU = dQ + h\,dn + 0$

2nd Law: $dS = \frac{1}{T}dU - \frac{\mu}{T}dn + \frac{A}{T}d\xi$

$$dS = \frac{Q}{T} + s\,dn + dS_{in}$$

$$\mu = h - Ts \qquad P_s = \dot{S}_{\dot{m}} = \frac{A}{T}\dot{\xi} \geq 0$$

Eckart-Onsager: $\Delta\ \dot{\xi} = \alpha\ \ n, \xi, T\ \ A + O\ \ A^2$

Equations of State: $\Delta\mu = F_{20}\Delta n + F_{11}\Delta\xi$

$$-A = F_{11}\Delta n + F_{02}\Delta\xi$$

*

$\Delta\mu\ \ t\ = \mu - \mu_0 \rightarrow \Delta n\ \ t\ = n - n_0\ ,\ \Delta\xi\ \ t\ = \xi - \xi_0\ ,\ A = A\ \ t\ \rightarrow 0!$

Stimulus Adsorption Structure Equilibrium

Hydratization Process of Proteins (System: P, w(a))

Stimulus : $\Delta\mu = \mu\ p,T,\ldots\ - \mu_0$

Adsorption: $\Delta n = n\ t\ - n_0$

Structure : $\Delta\xi = \xi\ t\ - \xi_0$ ... adsorption sites

$$\tau_n \Delta\dot{\mu} + \Delta\mu = E\ \Delta n + \tau_\mu \Delta\dot{n}$$ (Poynting, Elastic Relax.)

* $\tau_n^{-1} = \alpha F_{02} > 0\ ,\ E = F_{20} - \frac{F_{11}^2}{F_{02}} \geq 0\ ,\ \tau_\mu^{-1} = \left(F_{02} - \frac{F_{11}^2}{F_{20}} \right) \alpha > 0$

$\tau_n < \tau_\mu$

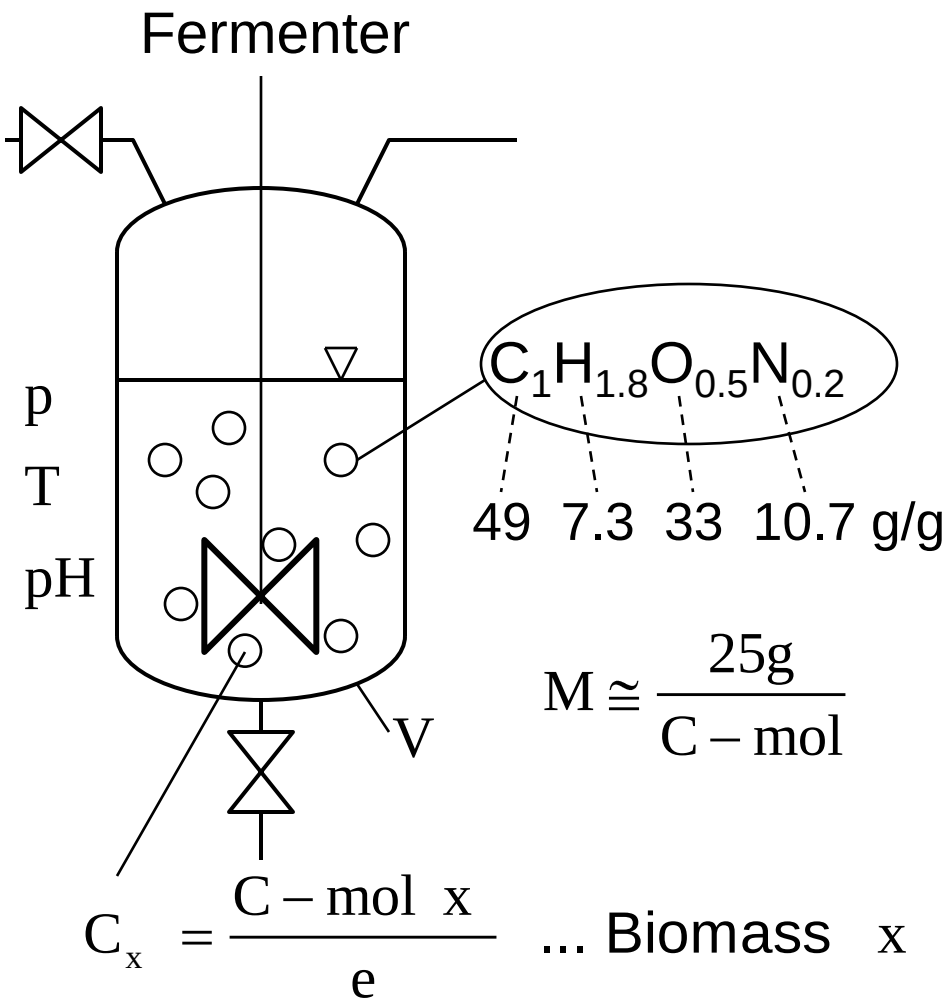
Adsorption Process

$$\Delta n\ t\ = \frac{1}{\tau_\mu E} \int_0^t ds \left[\Delta\mu\ s\ + \tau_n \Delta\dot{\mu}\ s\ \right] e^{-\ t-s\ /\tau_\mu} ds$$

Protein structure / Adsorption sites

$$\Delta\xi\ t\ = \frac{1}{F_{11}} \left\{ \Delta\mu - \alpha F_{20} \int_0^t ds \left[\Delta\mu\ s\ + \tau_n \Delta\dot{\mu}\ s\ \right] \right\} e^{-\ t-s\ /\tau_\mu} ds$$

5A. Metabolism of Living Bacteria*



Example (Yeast)

Genes	5000
Metabolites	1000-5000

Concentration*) $0.1-10\ \mathrm{m\,mol}$

Turn over time

$$\frac{\text{Concentration}}{\text{Reaction rate}} = 1-10\ \mathrm{s}$$

*) Osmotic pressure limited. Avoiding byproducts and byreactions.

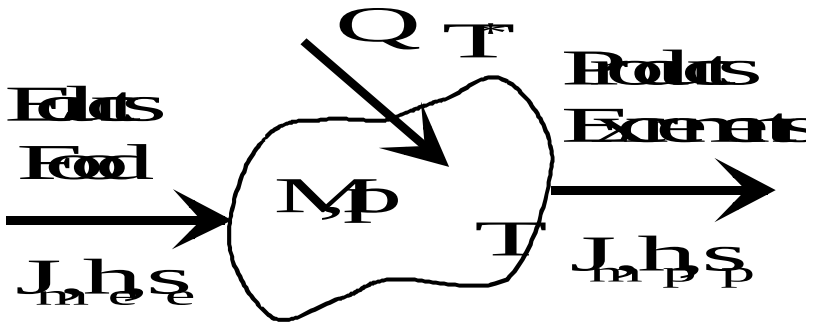
*Microbiothermodynamic system, Microbioreactor

Bacteria Stylonychia (Wimpertierchen / Eyelash bacteria)

Mesoscopic Biofluids / Bacterial Solutions

Exergy Analysis of Microbioreactors (MBRs)

Stationary States



$$1^{st}\ \text{Law:}\ \dot{U} = (h_e - h_p)J_m + \dot{Q} = 0 \tag{1}$$

$$2^{nd}\ \text{Law:}\ \dot{S} = (s_e - s_p)J_m + \frac{\dot{Q}}{T^*} + P_s = 0 \tag{2}$$

$$\text{Exergy:}\ \dot{E} = (e_e - e_p)J_m + \left(1 - \frac{T^*}{T}\right)\dot{Q} + P_{ex} = 0 \tag{3}$$

$$e_i = h_i - h_i^* - T^*(s_i - s_i^*), \quad i = e, p \tag{4}$$

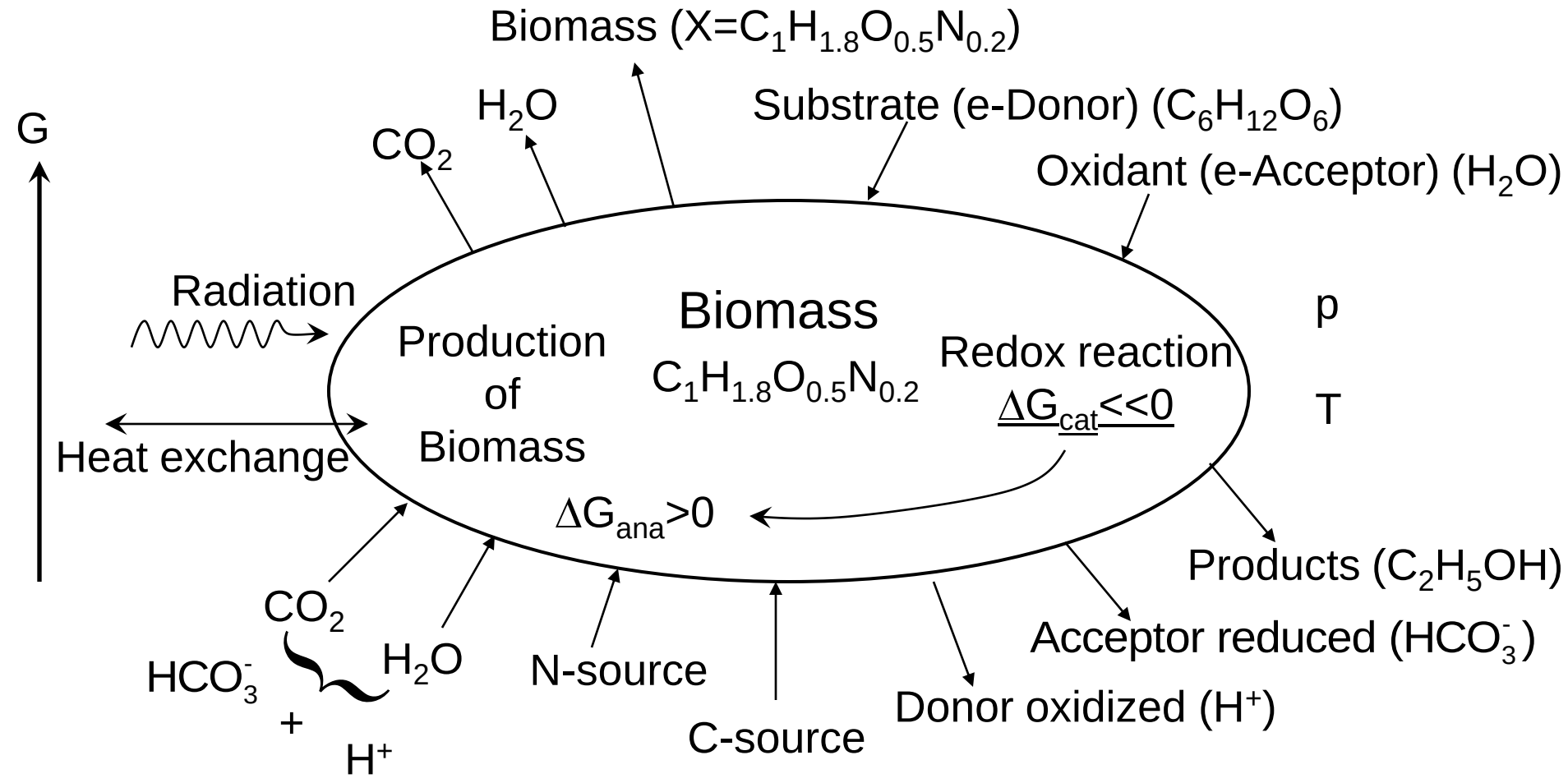
(1–3) $$\frac{e_p - e_e}{h_p - h_e} \le 1 - \frac{T^*}{T} \tag{5}$$ irr. cell / rev. cell $\rightarrow$

$$e_p \le e_e + \left(1 - \frac{T^*}{T}\right)(h_p - h_e) \tag{6}$$

COP of MBRs: $$\eta_{BR} = \frac{e_p}{e_{p\max}} \overset{(6)}{=} \frac{e_p}{e_e + \left(1 - \frac{T^*}{T}\right)(h_p - h_e)} \le 1 \tag{7}$$

(5-7): All bacteria, all metabolisms, any temperature and pressure!

Microbial Growth System



Anabolism + Catabolism (Free Entalpy)

5B Thermodynamic Limits of Life

Allometry

Metabolic Rate

$$\Gamma = a\ \ T, T_0\ \ \ M^{\gamma}$$

$$a \cong (1-2)\,\mathrm{mW/g}$$

$$\frac{2}{3} < \gamma \leq 1$$

$$\gamma \cong \frac{3}{4}$$

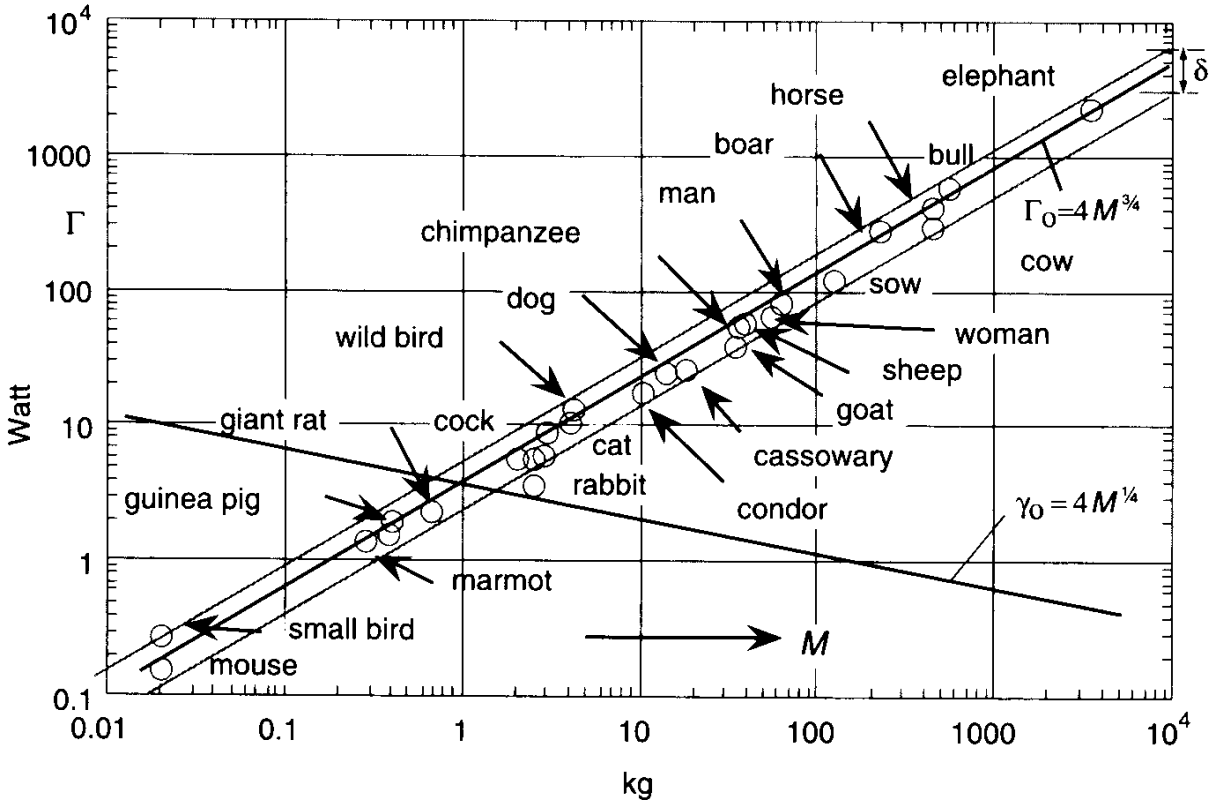


Figure A3
Metabolic rate of oxygen consumption based living systems. Mouse-Elephant-curve, B. Ahlborn, 2004. This curve also holds for bacteria (M $\cong 10^{-4}$ g).

Allometric Constant (a), Temperature Dependence

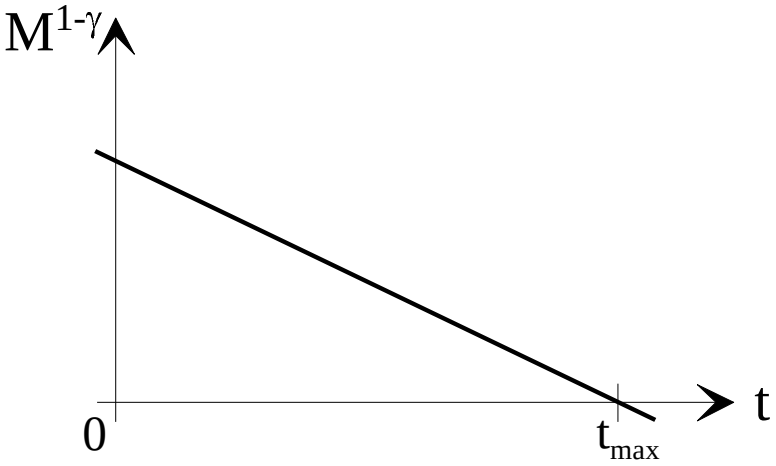


Figure A5
Mass reduction during autometabolism process of an organism.

1st Law $\dot{Q} = (h_e - h_p)J_m$

Allometry $\dot{Q} = a(T,T^*)M^{\gamma}$

$$a(T,T^*) = a_0(T - T^*)e^{-q^*/RT^*}$$

$$a\ (T = T^*) = 0$$

$$a\ (T\ ,\ T^* = 0) = 0\ \text{...all}\ (T)$$

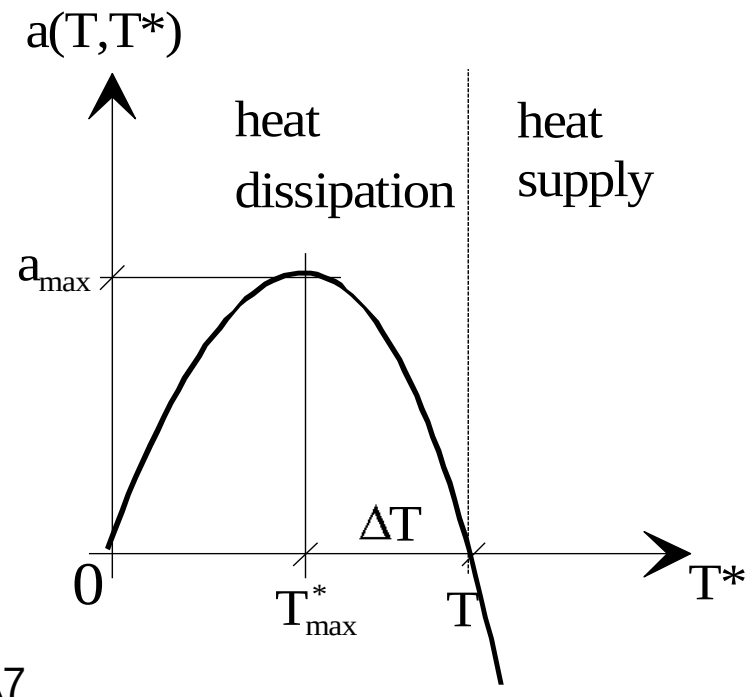


Figure A7
Dependence of the allometric constant (a) on the environmental temperature (T^*) of the bacteria.

Environmental Temperature for Maximum Metabolism at Given (M, T). System: Fig. A5

$$\Gamma = a\ T, T^*\ \ M^{\gamma}$$

$$a(T, T^*) \rightarrow \text{Max.}$$

$$\rightarrow\ \ T^* = T - \frac{q^*}{R} = T - \Delta T$$

$$\dot{Q}_{max} = a_0\, \Delta T\, e^{-\frac{\Delta T}{T^*}}\, M^{\gamma}$$

$$J_{max} = \dot{Q}_{max} \Big/ h_e - h_p$$

$$\frac{J_{max}}{M} = \frac{a_0 \Delta T}{h_e - h_p} M^{-1+\gamma} \cdot e^{-\frac{\Delta T}{T^*}}$$

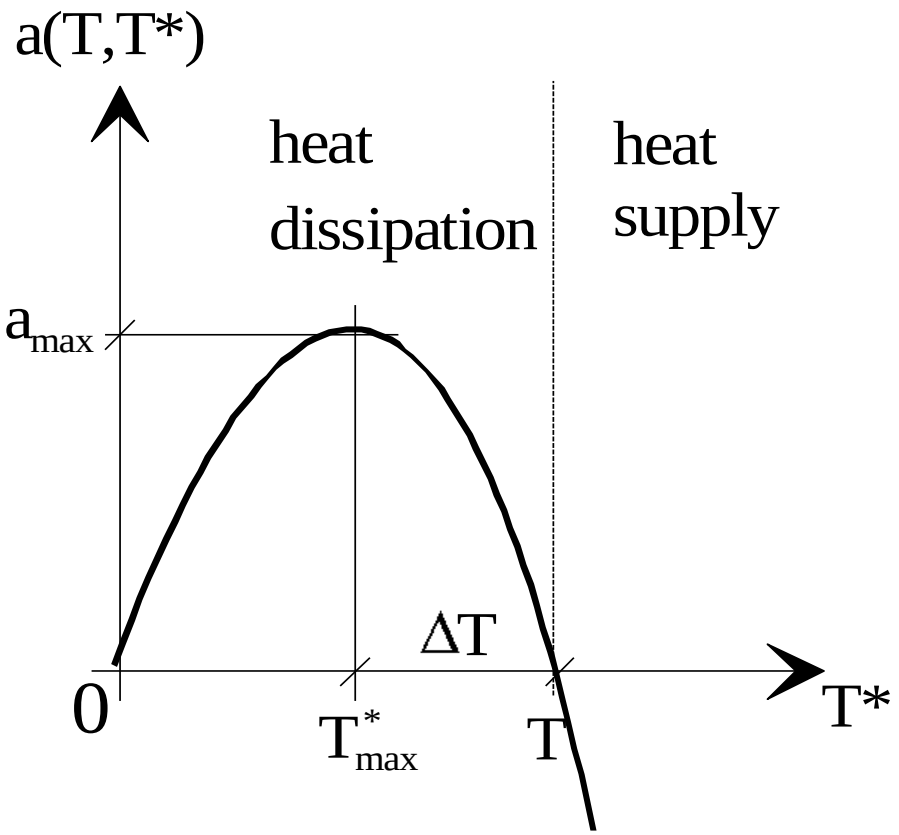
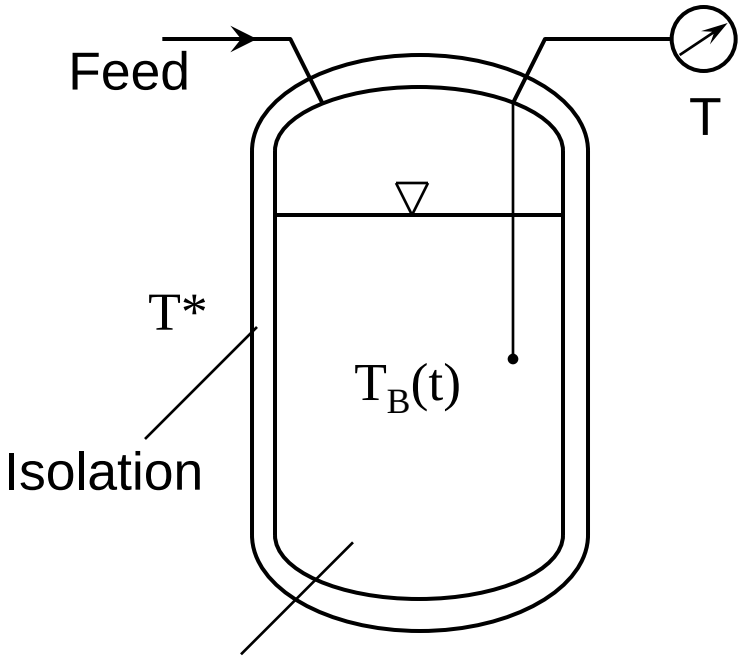


Figure A7
Dependence of the allometric constant (a) on the environmental temperature (T^*) of the bacteria.

6. Biocalorimetry
Bacterial Identification by
Caloric Measurements of Growth Processes (E5)

Adiabatic Calorimeter



Broth: Water (n_W)
Substrate (n_S)
Bacteria (n)
.....

Metabolic heat

$$dQ = C_B \; n,... \; dT_B \qquad (1)$$

$$Q(t) = C_B \; T_B(t) - T_0 \quad (1A)$$

Metabolic generation of heat:

$$dQ \cong -dn_S \qquad (2)$$

$$Q(t) = K\left[n_{S_0} - n_S(t) \right] \qquad (2A)$$

$$n_S(t) = n_{S_0} - \frac{Q(t)}{K} \qquad (2B)$$

Bacterial Identification by Caloric Measurements

Bacterial growth process:

$$dn \cong n_S n\, dt \qquad (3)$$

$$\frac{dn}{n} = A\, n_S(t)\, dt \qquad (3A)$$

(2B) $$\frac{dn}{n} = A\left[n_{S_0} - \frac{Q(t)}{K} \right] dt$$

(1A) $$n(t) = n_0 \exp\left\{ A \int_0^t \left[n_{S_0} - \frac{C_B}{K}\, T_B(s) - T_{B_0} \right] ds \right\} \qquad (3B)$$

Bacterial population

Measurement

Process model: Monod

$$n(t) = n_0 + n_\infty - n_0 \frac{b\, t^{\alpha}}{1 + b\, t^{\alpha}} \qquad (4)$$

$$(3B, 4) \rightarrow \alpha, b = \frac{1}{t_B}$$

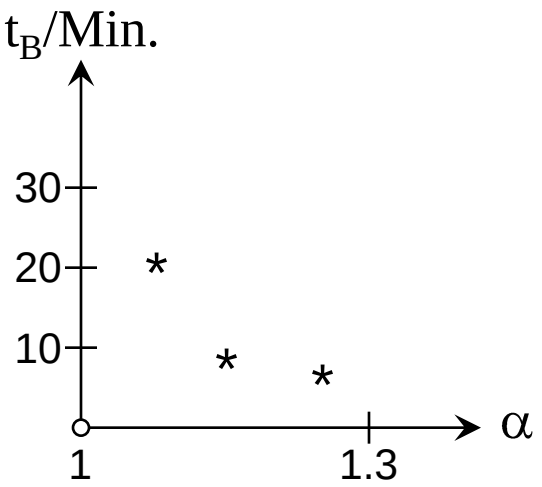
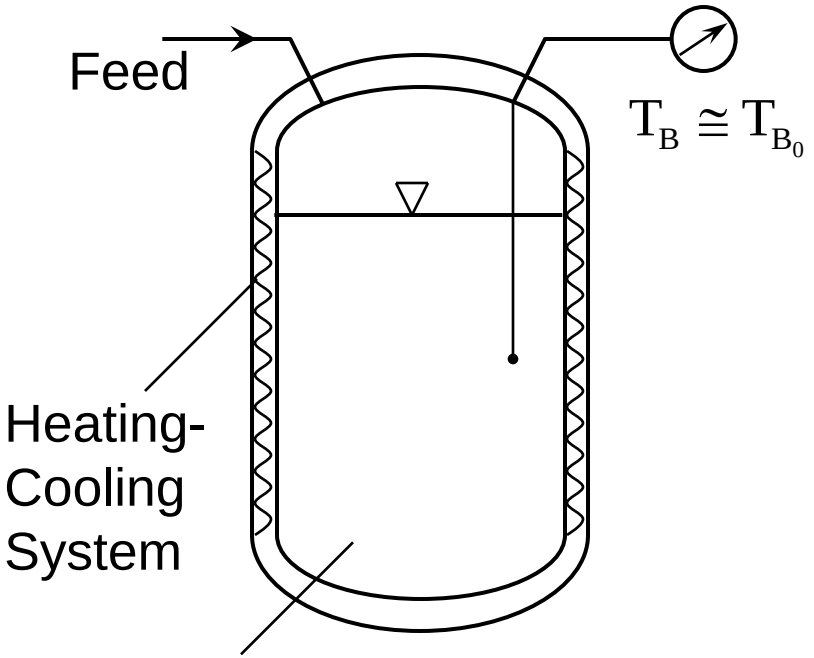


Diagram of characteristic parameters (α, t_B=1/b)

Bacterial Identification by Caloric Measurements of Growth Processes

Isothermal Calorimeter



Broth: Water (n_W)
Substrate (n_S)
Bacteria (n)
.....

Metabolic generation of heat:

$$dQ \cong -dn_S \qquad (2)$$

Compensational heat (Peltier)

$$dQ_C = \Pi I^2 dt \qquad (5)$$

Isothermal condition

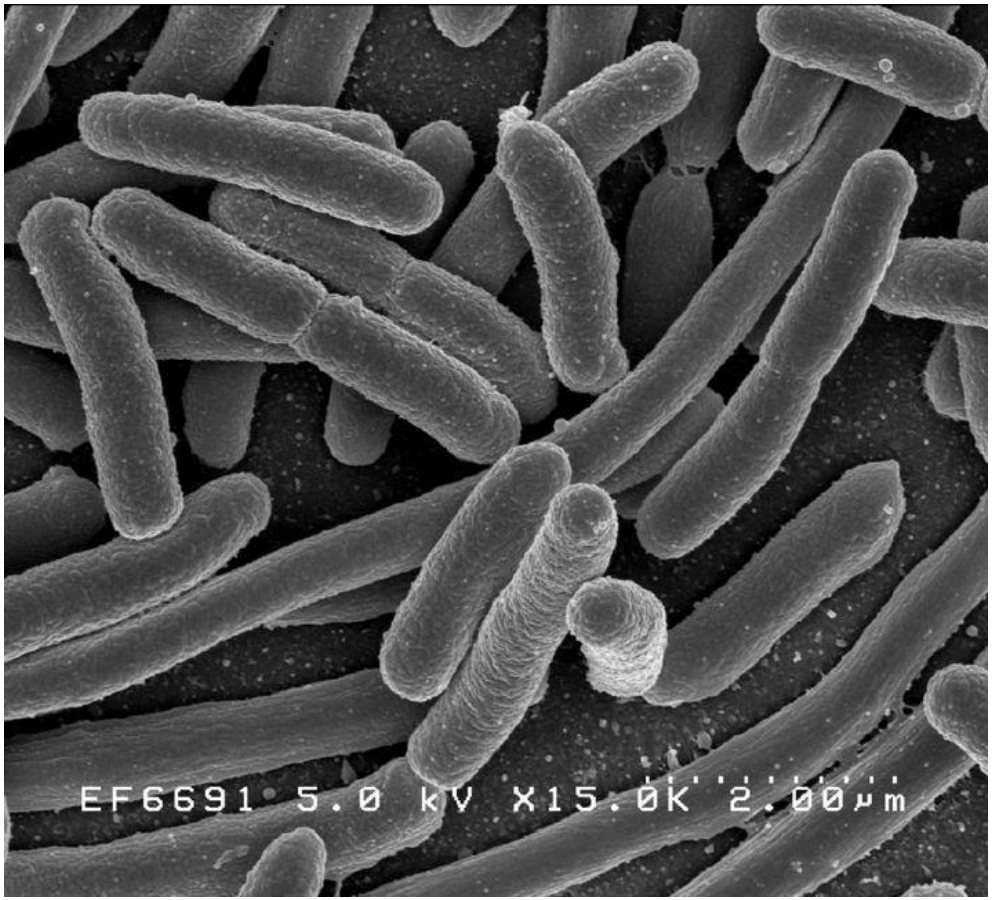
$$T_{B_0}: \quad 0 = dQ + dQ_C \quad (6)$$

Bacterial growth measurement (3)

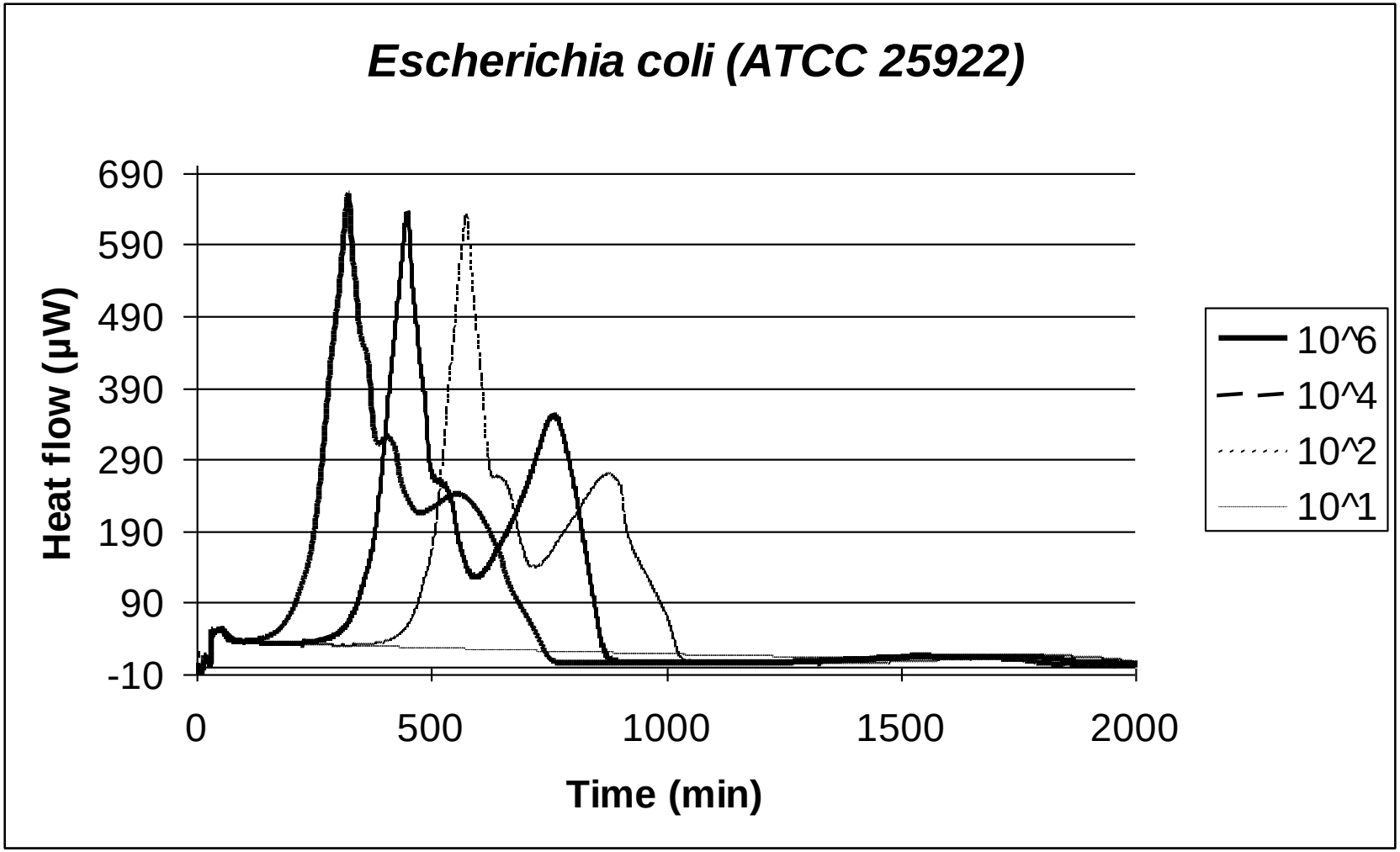
$$n(t) = n_0 \exp\left\{ A \int_0^t \left(n_{S_0} + \frac{Q_C(s)}{K} \right) ds \right\}$$

Model (monod)

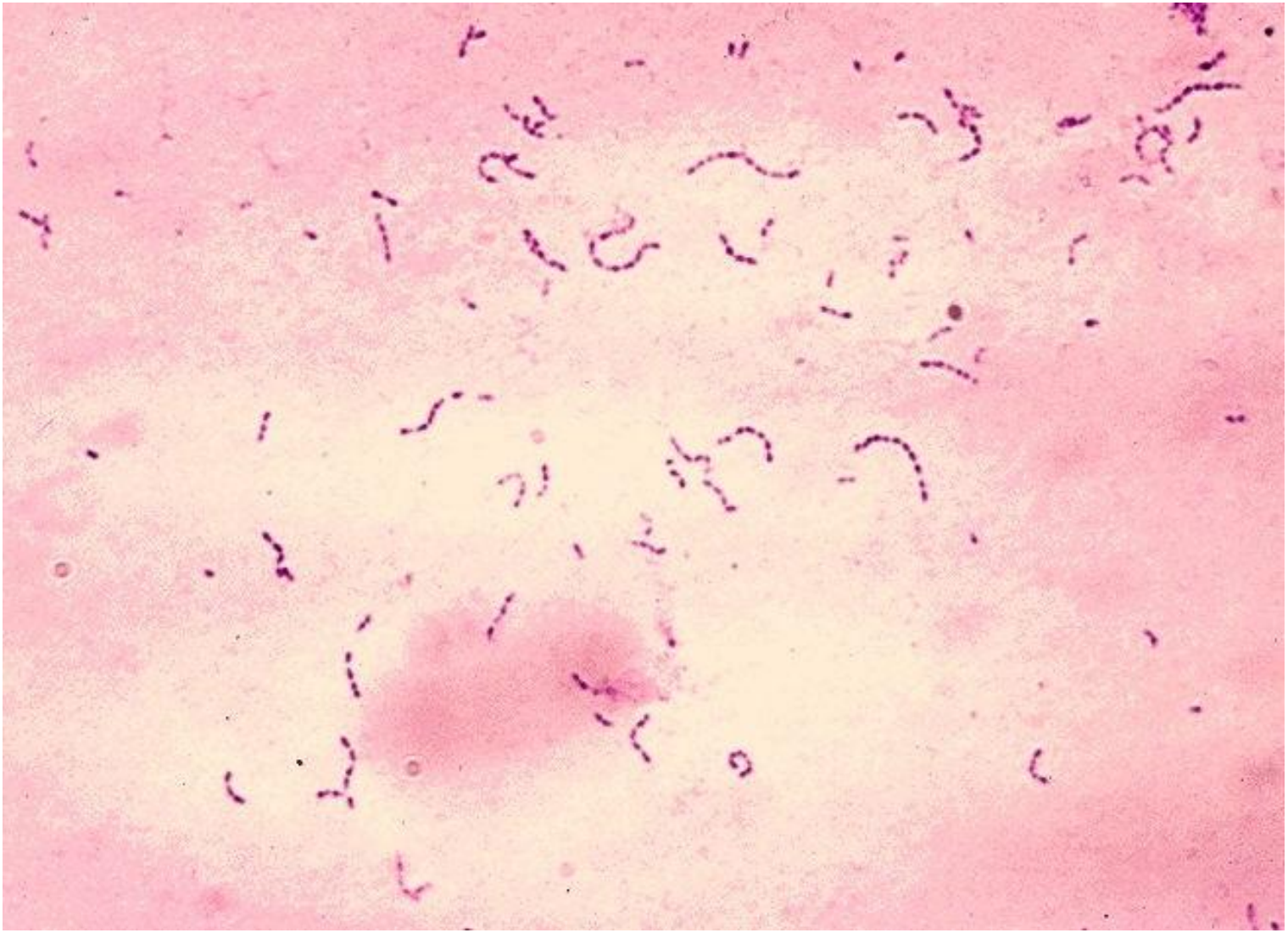
$$(4) \quad n(t) = n_0 + (n_\infty - n_0) \frac{(bt)^\alpha}{1+(bt)^\alpha} \rightarrow (\alpha, b)$$



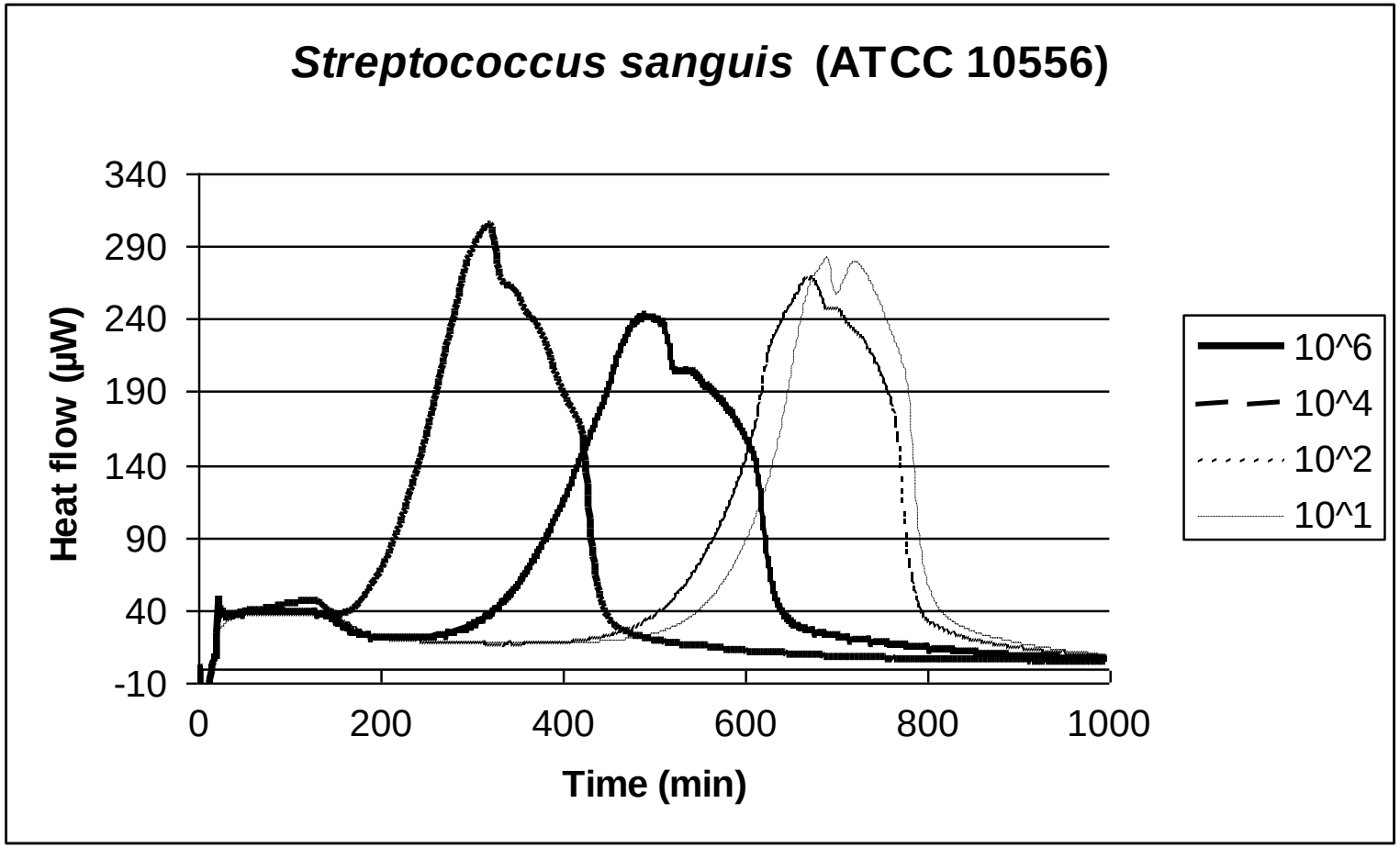
Bacteria Escherichia coli, Th. Escherich (1919)



Ref. A.Trampuz et al., Biocalorimetry..., Trans-2007-0018.R2



Bacteria Streptococcus Mutans (Karies), Clarke (1924)

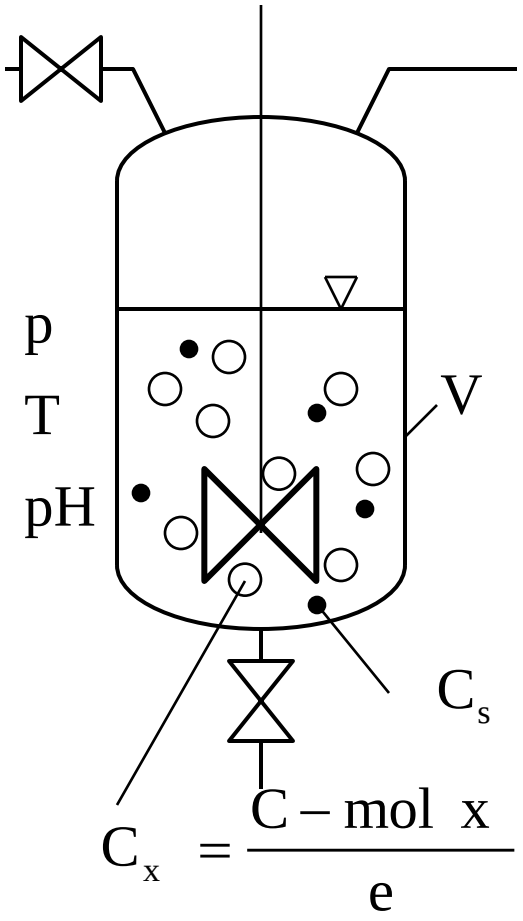


Ref. A.Trampuz et al., Biocalorimetry..., Trans-2007-0018.R2

6.Bioreactors

Microbial Growth at Constant Substrate Concentration

Fermenter



Rate equation

$$dC_x = \mu_x C_x dt$$

Growth rate*)

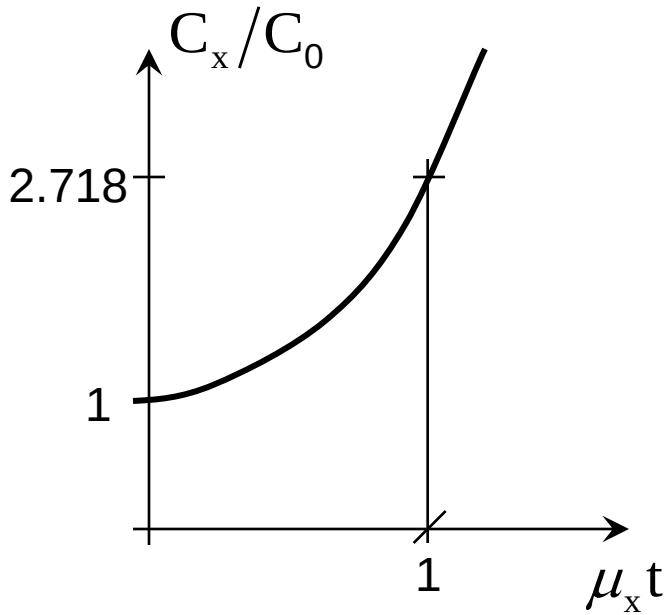
$$\mu_x = \frac{C-mol\ x}{C-mol\ x \cdot h}$$

$0.001-2$

Microbial growth

$$C_x\ t = C_0\, e^{\mu_x t}$$

$$\mu_x = const = \frac{1}{\tau}$$



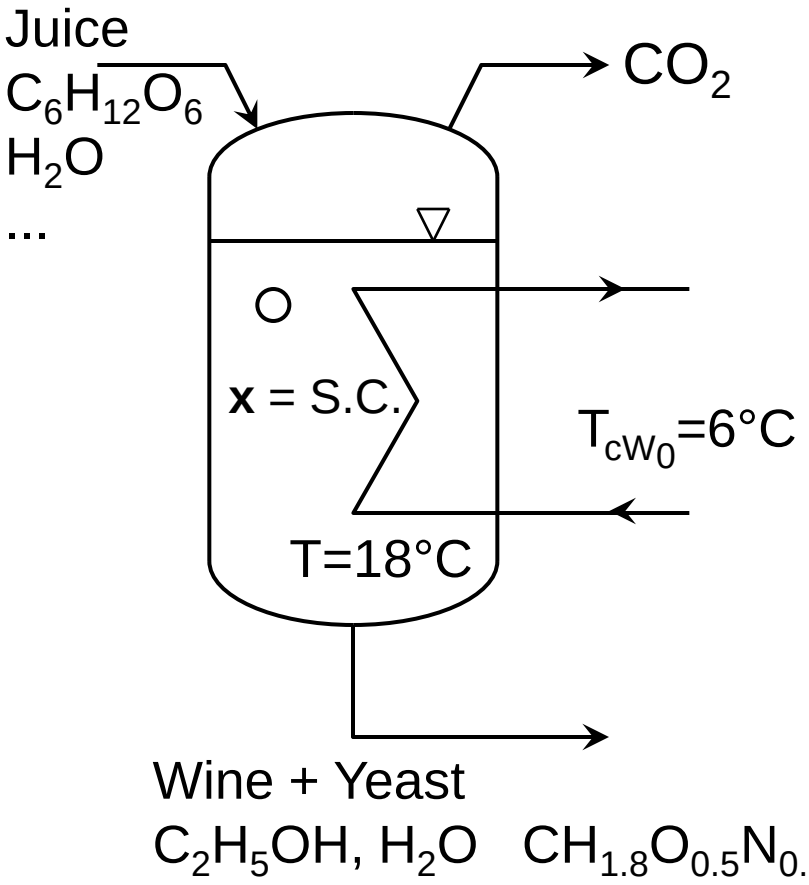
$C_s = \frac{mol\ s}{e}$ ... Substrate s

$C_x = \frac{C-mol\ x}{e}$ ... Biomass x

*) Limited by $\bar{e}$-transport

capacity in cell membranes: $3\,mol\ \bar{e}\ /C-mol\ x\ h\quad 298\,K$

Wine Fermentation, Heat Production, Cooling Process (1)
Problems: Oxygen, Pressure, pH-Value



Catabolic reaction

$$- C_6H_{12}O_6 + 2C_2H_5OH + 2CO_2 = 0 \quad (C)$$

Anabolic fermentation reaction

$$a\ C_6H_{12}O_6 + b\ NH_3 + c\ H_2O + d\ CO_2 \quad A)$$
$$+ e\ C_2H_5OH + 1 \cdot CH_{1.8}O_{0.5}N_{0.2} = 0$$

x = S.C.: Saccharomycae cervisiae
Wine yeast (Weinhefe)

Anabolic growth (Experiment)

$$Y_{GLUX} = 0.057 \frac{\text{C-mol x}}{\text{C-mol GLU}}$$

Wine Fermentation, Heat Production, Cooling Process (2)

Biological parameters

$$Y_{GLUX} = 0.057 \frac{C-mol\ x}{C-mol\ GLU}$$

Growth rates

$$\mu_x = 0.05 \frac{g}{g\,h} ...18°C$$

$$\mu_x = 0.34 \frac{g}{g\,h} ...30°C$$

Molar mass (including ash)

$$M_x = 26 \frac{g}{C-mol\ x}$$

Enthalpy of combustion

$$\Delta h_x = -472 \frac{kJ}{C-mol\ x}$$

Initial yeast concentration

$$n_{x0} = 0\,C-mol\ x/l$$

Oenological parameters

Juice

$$c_{GLU_0} = 210 \frac{g}{l}$$

$$M_{GLU} = 180 \frac{g}{mol}$$

$$\nu_{GLU_0} = 1.167 \frac{mol}{l}$$

$$\nu_{GLU_\infty} = 0$$

$$\rho_J = 1.0\ kg/l = \rho_{Wine}$$

$$c_{pJ} = 4.186 \frac{kJ}{kg\,K} = c_{pWine}$$

Technical parameters

$$V = 10000\,l$$

$$T = 18°C$$

$$T_{cW_0} = 6°C$$

Heat transfer

$$k = 200 \frac{W}{m^2 K}$$

Wine Fermentation

Problems

1. Stoichiometry of anabolism
   Heat production
2. Stoichiometry of catabolism
   sugar → alcohol
3. Pressure dependence
4. Heat balance of reactor
5. Maximum heat production rate
6. Heat exchange area
   Tube length, cooling water flow

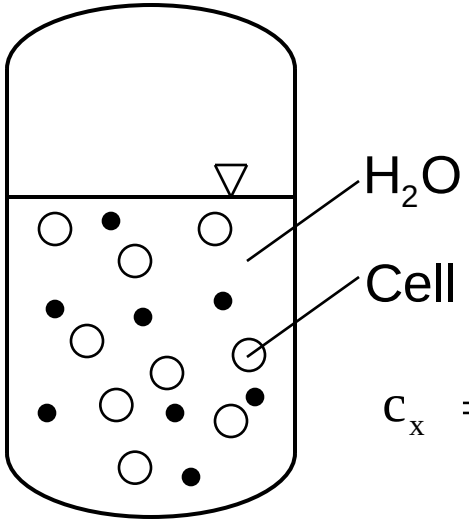
Thermodynamic Data

Heat of combustion

(25°C, 1atm, pH=7)

Glucose ($C_6H_{12}O_6$)	–2813.6 kJ/mol
Ethanol (C_2H_5OH)	–1356.8 kJ/mol
Biomass ($CH_{1.8}O_{0.5}N_{0.2}$)	-475kJ/mol
CO_2, NH_3, H_2O	~~0~~ kJ/mol

Phenomenological Kinetics of Cell Death (Sterilization Processes)



$$c_x = \frac{C - mol\ x}{l}$$

Cell death $\simeq$ Enzyme deactivation

loss of viability

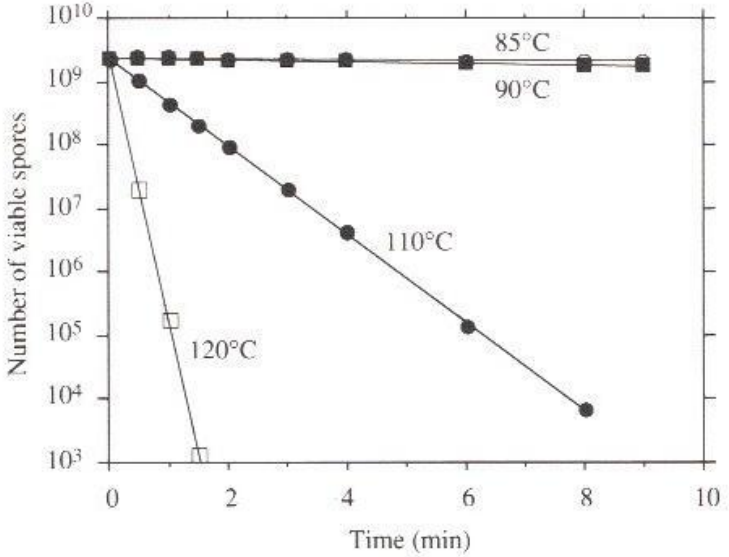
$$\dot{c}_x = -k_d c_x \qquad 1$$

$$k_d\ T = k_{d_0} e^{-\frac{E_d}{R}\left(\frac{1}{T}-\frac{1}{T_0}\right)}$$

$$E_d = 250 - 300\ kJ/mol$$

$$1 \quad c_x\ t = c_{x_0} e^{-k_d t}$$

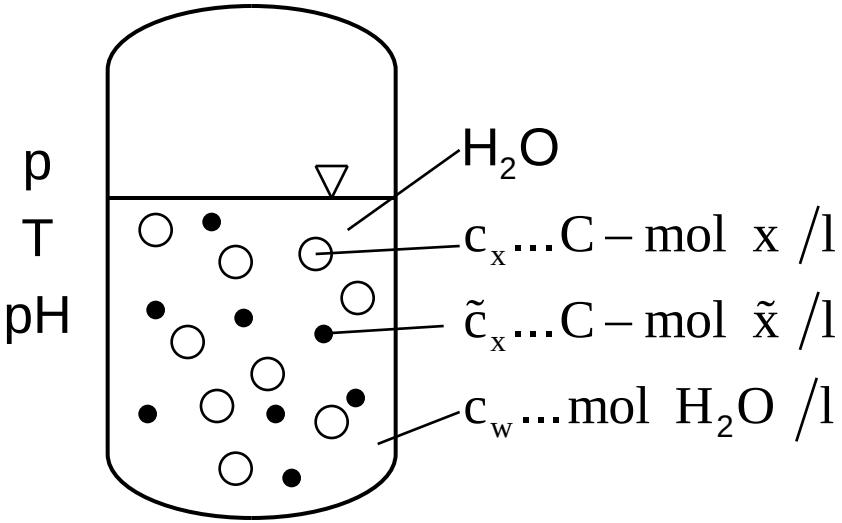
$$\ln c_x = \ln c_{x_0} - k_d t \qquad 2$$



Thermal death of Bacillus subtilis spores.

T/°C	85	90	110	120
k_d/min^{-1}	0.012	0.032	1.60	9.61

Thermodynamics of Cell Death Processes*) (T-Dependence)



Death reaction: $x \rightarrow \tilde{x}$

$$G = G\ n_x, \tilde{n}_x, n_w, T, p$$

$$p,T: dG = \mu_x dn_x + \tilde{\mu}_x d\tilde{n}_x \qquad 1$$

Mass balance (C-Atoms)

$$d\tilde{n}_x = -dn_x \left| \frac{1}{V} \right.$$

$$\tilde{c}_x = \tilde{c}_{x_0} - c_x - c_{x_0} \qquad 2$$

*) Analogy: Radioactive gas.

1,2 $$dg = \overbrace{\mu_x - \tilde{\mu}_x}^{\text{Force}}\ \overbrace{dc_x}^{\text{Flux}} < 0 \quad \ldots 2^{nd}\ \text{Law}$$

$$\dot{c}_x = F\ \mu_x - \tilde{\mu}_x \qquad 3$$

1st order kinetics

$$\dot{c}_x = -k_d c_x \qquad 4$$

Thermostatics of ideal solutions

$$\tilde{\mu}_x = \tilde{\mu}_{x_0}\ T,p\ + RT \ln \tilde{x}_x\ ,\ \tilde{x}_x = \frac{\tilde{c}_x}{c_x + \tilde{c}_x + c_w}$$

$$\mu_x - \tilde{\mu}_x = \mu_{x_0} - \tilde{\mu}_{x_0} + RT \ln\left(\frac{c_x}{\tilde{c}_x}\right) \qquad 5$$

(2-5) $$\dot{c}_x = -k_d \frac{c_{x_0} \exp\left(\frac{\mu_x - \tilde{\mu}_x}{RT}\right)}{K + \exp\left(\frac{\mu_x - \tilde{\mu}_x}{RT}\right)} = -k_d c_x \qquad 6$$

$$K(p,T) = \exp\left(\frac{\mu_{x_0} - \tilde{\mu}_{x_0}}{RT}\right)$$

$$F\ \mu_X - \tilde{\mu}_X$$

Production of Biomacromolecules*

Upstream Processing
25 C, $: 20%-30%

Genetic engineering
Genomics, Proteomics

Microbiology
Bacteria, Fungi, Cells

Fermentation

Cell harvesting

Selection of
Protein encoding
Gene

Selection of
Microbioreactor

Cell production

Downstream Processing
-200C – 150C, $:70%-80%

Cell disruption

Centrifugation

Ultracentrifugation

Chromatography
High resolution
Purification

Product / Formulation (pH)

*Recombinant proteins, DNAs, Ref.Tosoh Bioscience GmbH, Voet&Voet, Biochemistry

Problems in Downstream Processing of Biological Fluids

Parameters	Non-Biological Fluids	Downstream Processing Fluid
Number of Compounds	Low	Very high (>1000)
Pure State Data	Available	Difficult
Interactions	similarities	Whole spectrum (Coulomb, v.d.Waals)
Molecular Weight	comparable	Very different, from low to very high
Model Description	Possible with semi-empirical models	No model
Prediction of a a Unit Separation	Possible	Presently not Possible

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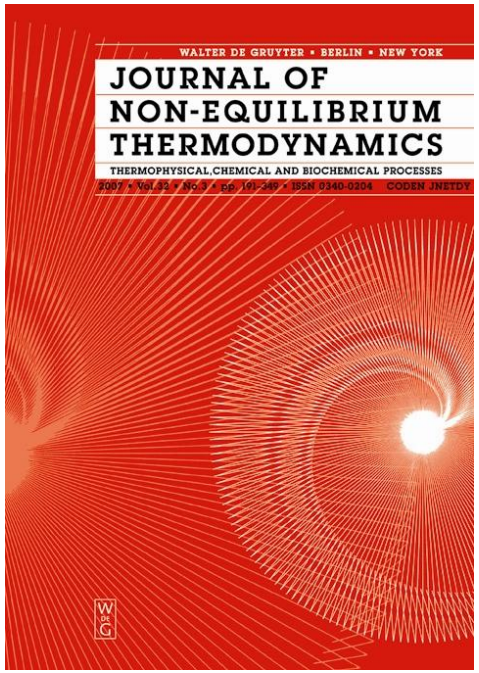

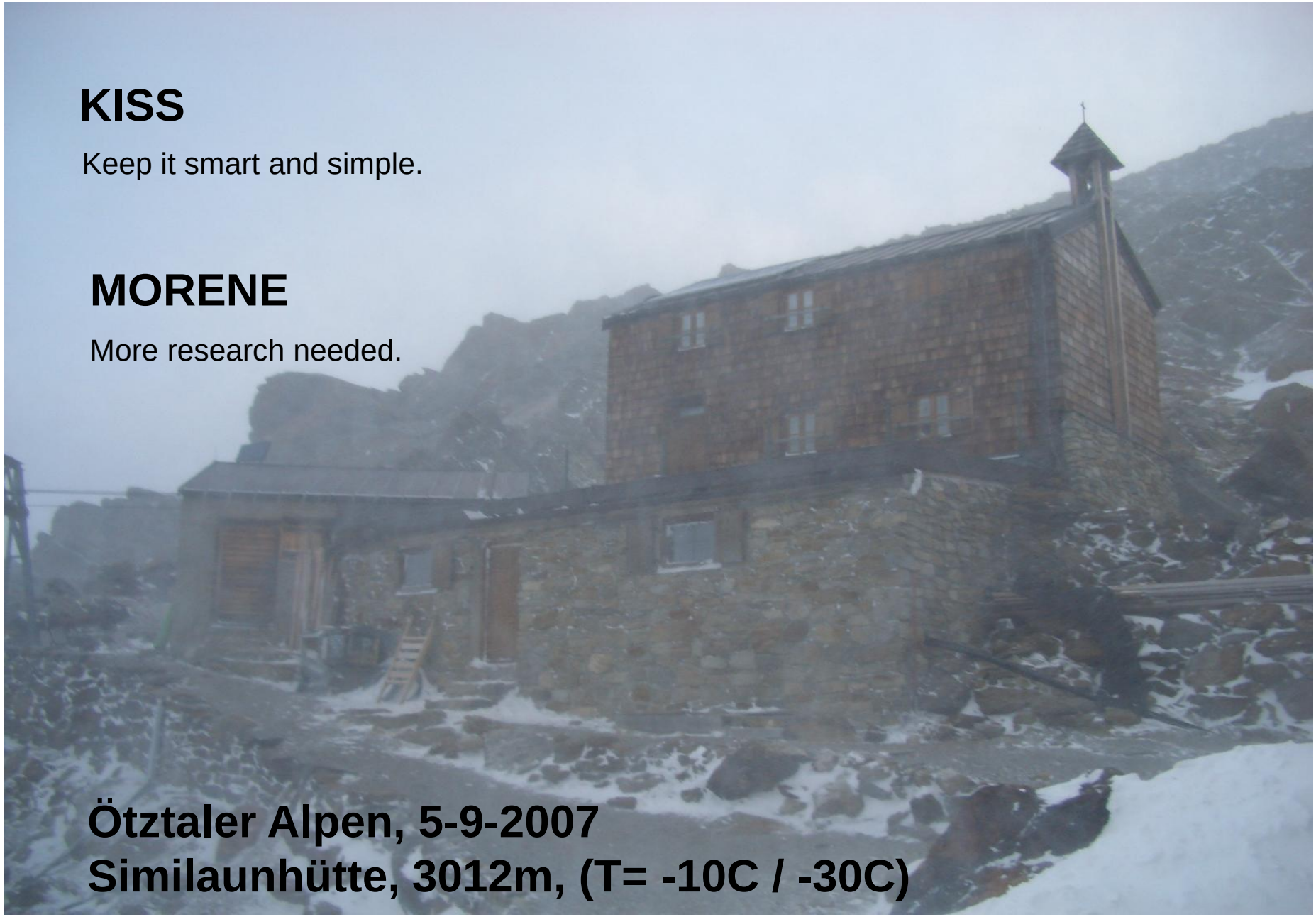
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More research needed.
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