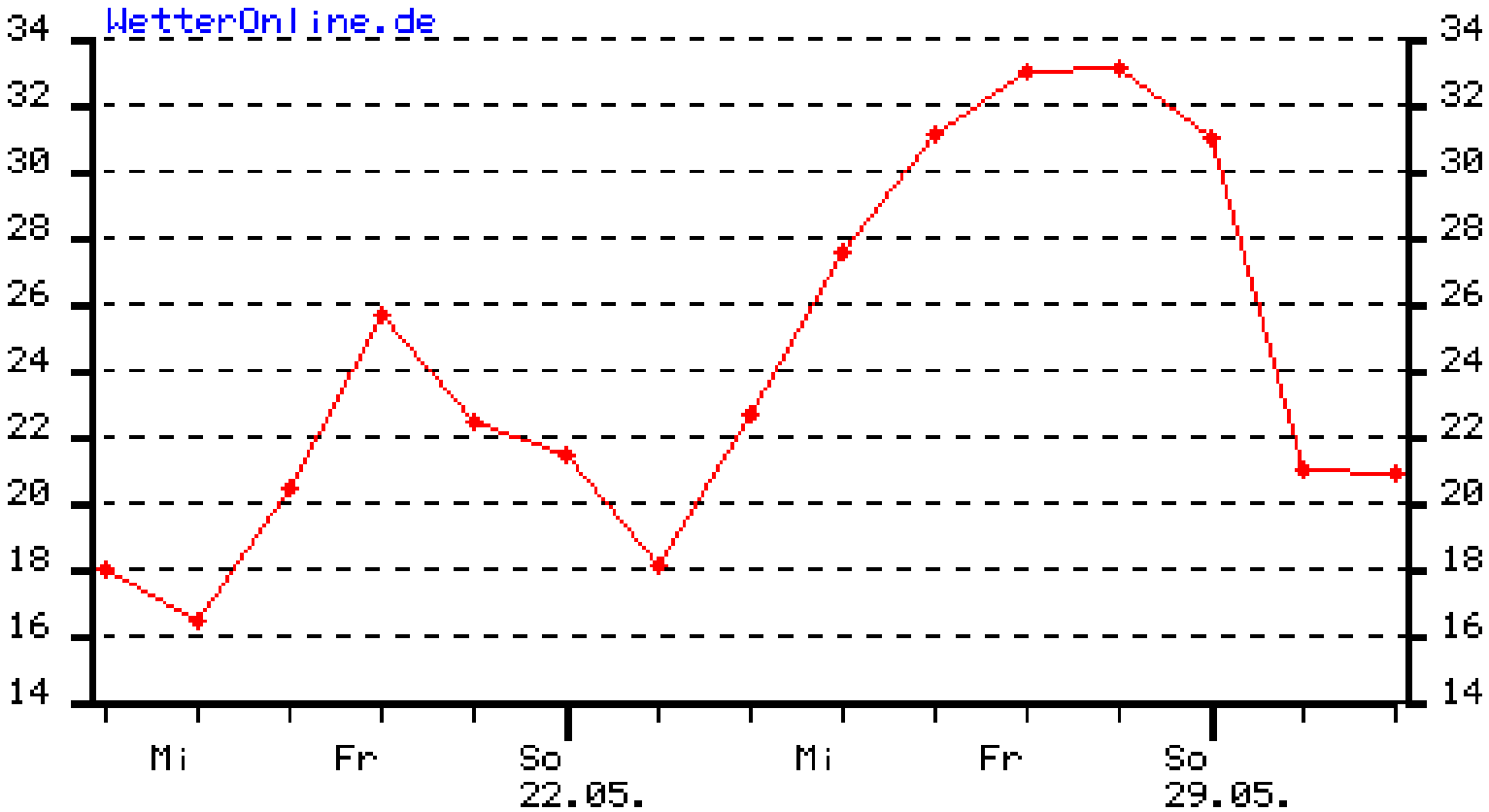
# Differential Scanning (micro)Calorimetry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# Outline

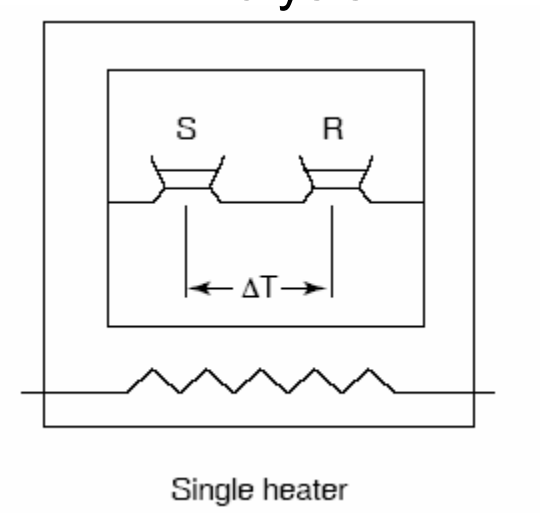
- DSC as method of thermal analysis (definitions, principle)
- Instrumentation
- DSC outcome (thermograms, determined parameters)
- Applications
  - Pharmaceuticals
  - Proteins
  - Lipids

# Thermoanalytical methods

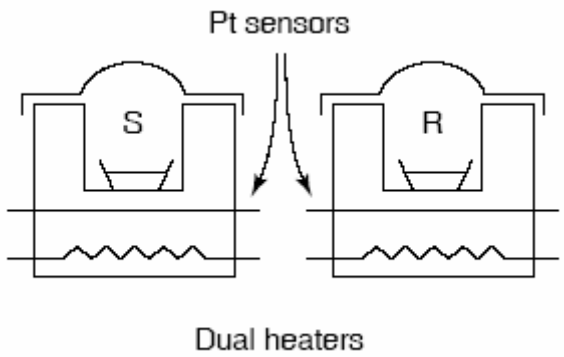
| *Method* | *Symbol* | *Measure* |
|---|---|---|
| Thermogravimetry | TG(TGA) | Mass (change) |
| Derivative Thermogravimetry | DTG | 1st derivative of mass change |
| **Differential Thermal Analysis** | **DTA** | **Themperature difference vs. reference** |
| Simultane Thermal Analysis STA (combination of DTA & TG) | | see DTA & TG |
| **Differential Scanning Calorimetry** | **DSC** | **Heat flow difference of sample vs. reference** |
| Dynamic Differential Calorimetry | DDC | |
| Thermomechanical Analysis | TMA | Mechanical properties |
| Dynamic Mechanical Analysis | DMA | Visco-elastic properties |
| Dilatometry | - | Linear thermal expansion |
| Evolved Gas Analysis | EGA | Kind & amount of released gas |
| Thermomagnetometry | TM | Magnetic properties |
| Thermosonimetry | TS | Acoustic properties |
| Thermooptic Analysis | TOA | Optic properties |

# DTA vs. DSC

## Differential Thermal Analysis




## Differential Scanning Calorimetry



# DSC: definitions

**Heat flow** = heat/time = dq/dt

**Heating rate** = temperature increase/time=dT/dt

**Heat capacity** = C = heat flow/heating rate =dq/dT

Adiabatic conditions: $\mathbf{C_p} = (dq/dT)_p = dH/dT$

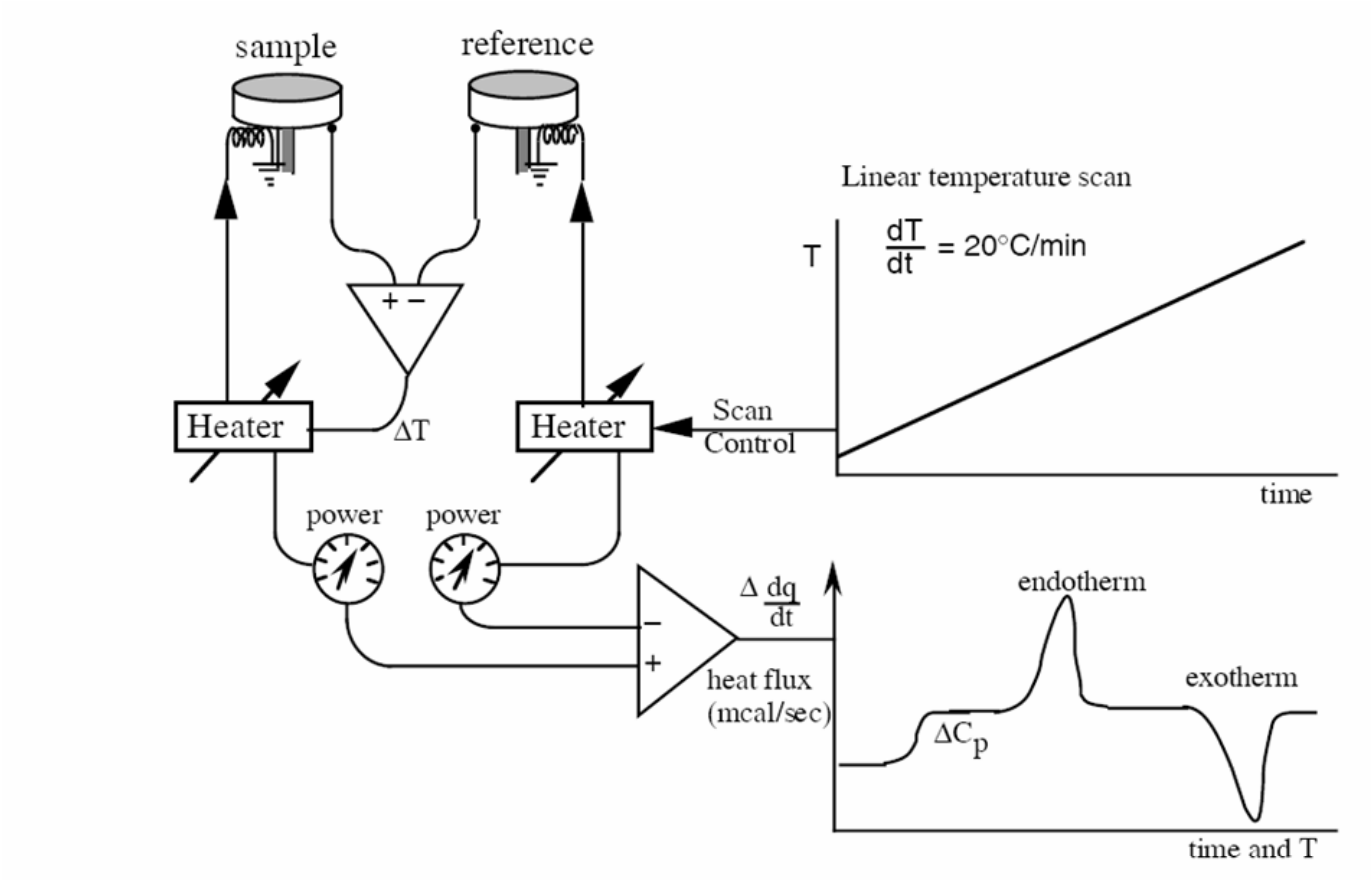
$\mathbf{\Delta C_p} = (C_p)_{sample} - (C_p)_{reference}$

$\mathbf{\Delta dH/dt} = (dH/dt)_{sample} - (dH/dt)_{reference}$

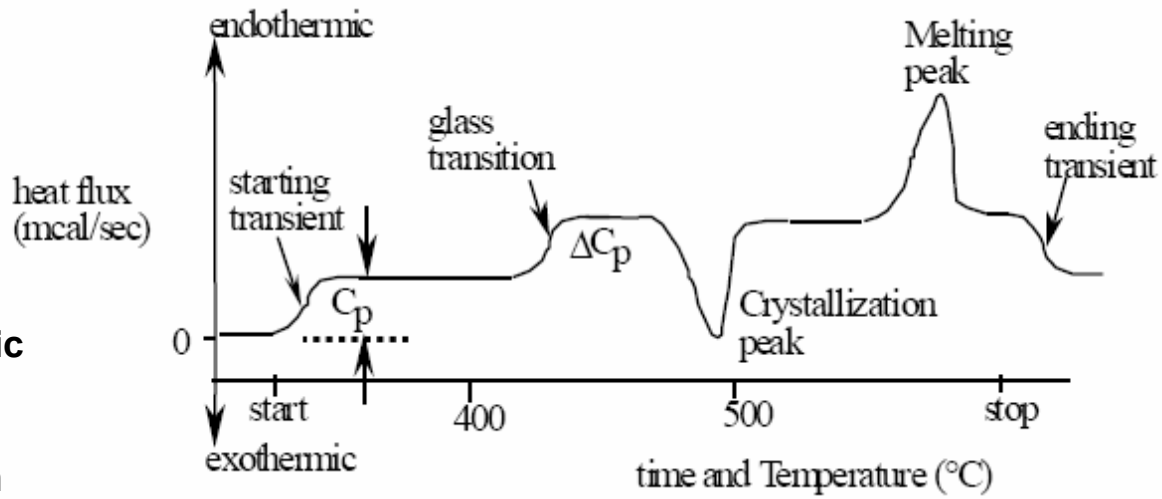
# Instrumentation



# DSC output


sample
reference
+ −
Heater
ΔT
Heater
power
power
Scan
Control
Linear temperature scan
T
dT/dt = 20°C/min
time
Δ dq/dt
heat flux
(mcal/sec)
endotherm
exotherm
ΔCp
time and T

# Transitions observed in DSC




**Endothermic**

Fusion
Vaporisation
Sublimation
Desorption
Desolvation
Decomposition
Reduction
Degradation
Glass transition
Relaxation of glasses

**Endothermic/Exothermic**

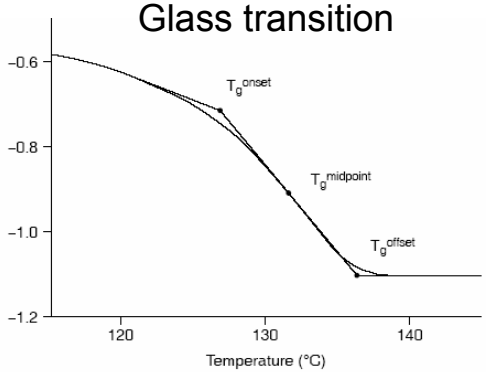
Decomposition
Degradation

**Exothermic**

Crystallisation
Condensation
Solidification
Adsorption
Chemisorption
Solvation
Decomposition
Oxidation
Curing of resins

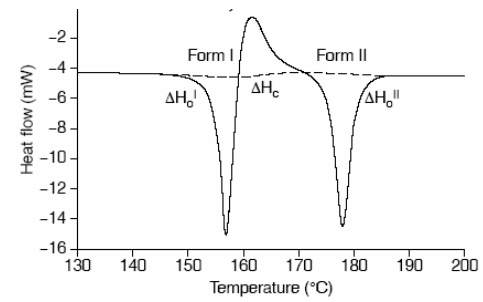
# DSC parameters

Glass transition

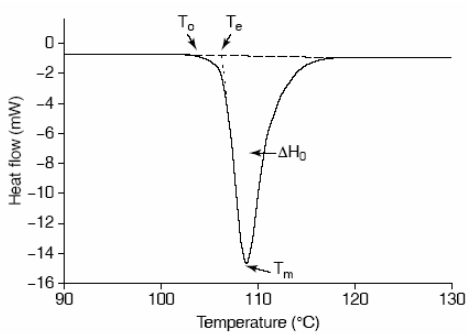



**$T_g$** – glass transition temperature
**$T_c$** – recrystallisation exotherm temperature
**$T_m$** – melting endotherm temperature
**$T_o$** – transition onset
**$T_e$** – transition extrapolated onset
**$\Delta T_{1/2}$** – sharpness of the transition
**$\Delta H_c$** – recrystallization enthalpy
**$\Delta H_o$** – melting enthalpy

Recrystallization




Melting transition



# DSC parameters

**$T_m$** – transiton maximum
**$\Delta T_{1/2}$** – sharpness of the transition
**$\Delta H_{cal}$** – transition ehthalpy

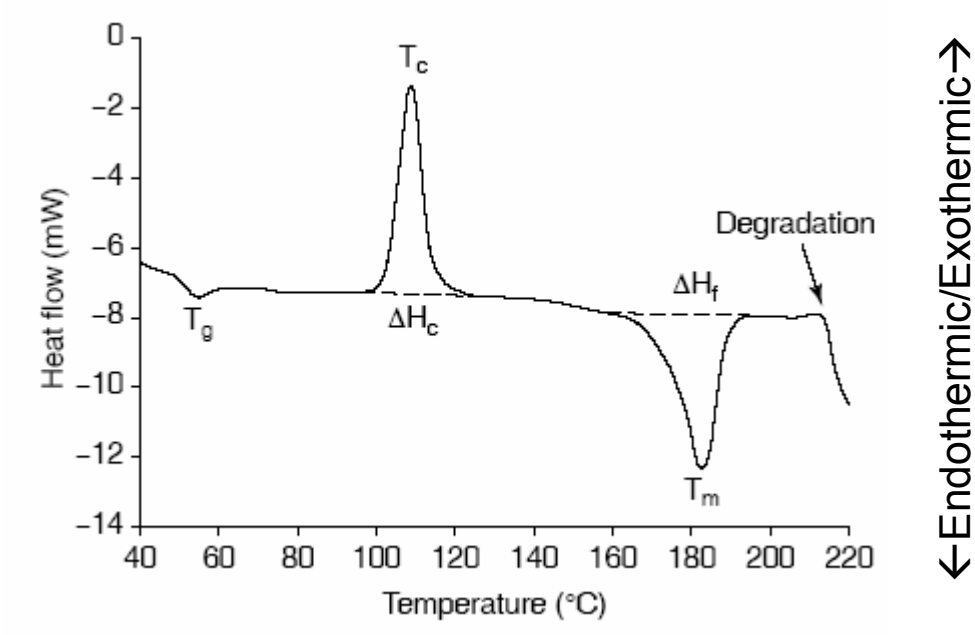
**$\Delta H_{cal}$** – directly measured

@$T_m$ $\Delta G=0 \rightarrow$ **$\Delta S$**= $\Delta H_{cal} / T_m$

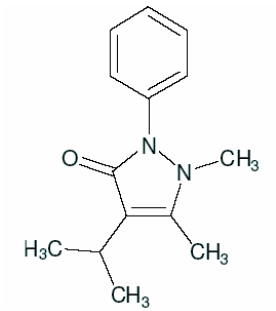
**$\Delta H_{vH}$** ~ $4RT_m^2 / \Delta T_{1/2}$

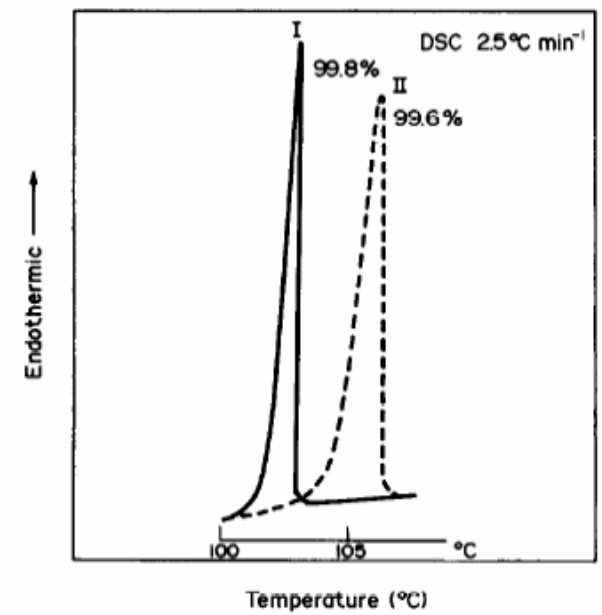
Cooperative Unit (**CU**)~ $\Delta H_{vH} / \Delta Hcal$

# DSC of glucose

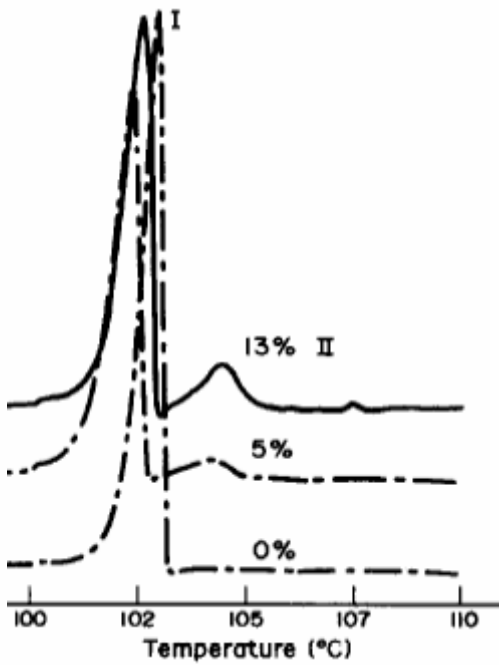


# DSC of prophenazone

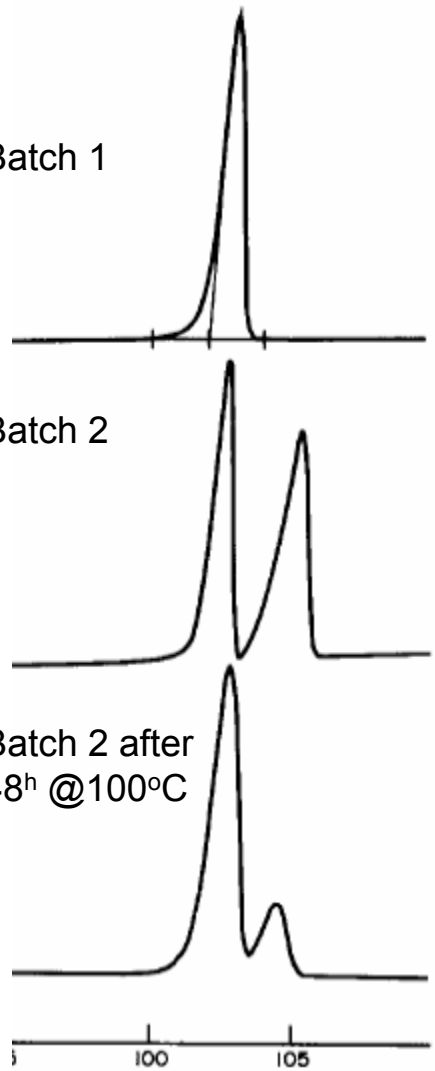



Different crystalites: I & II




Different batches



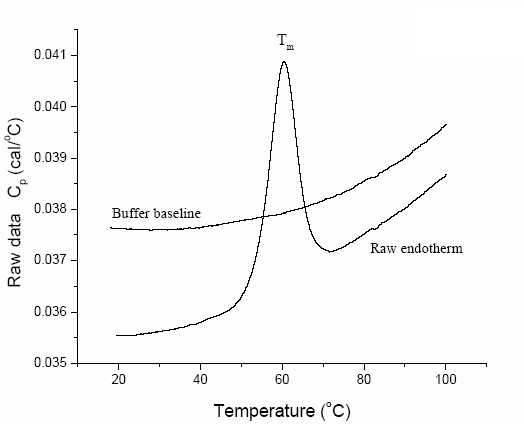
# DSC of pharmaceuticals

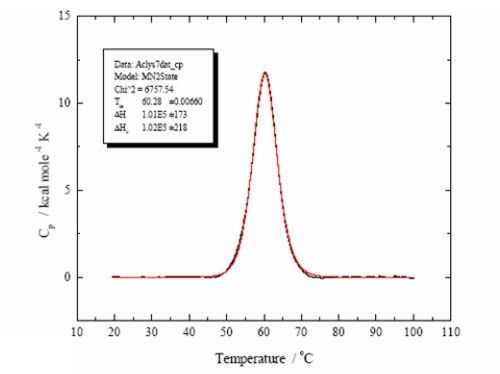
**Characterization of single components**
Purity
Quantification of crystallinity
Polymorphism
Degradation, decomposition, stability determination
Glass transitions

**Characterization of multicomponent mixtures**
Drug-excipient compatibility
Glass transitions in mixtures
Water & hydrates

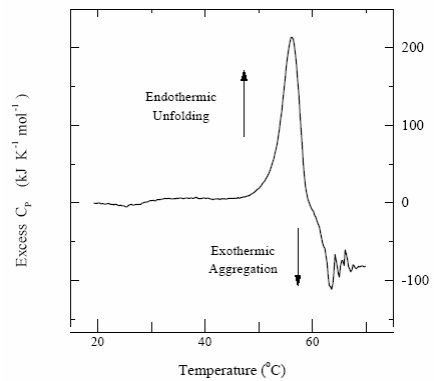
# DSC of proteins: denaturation

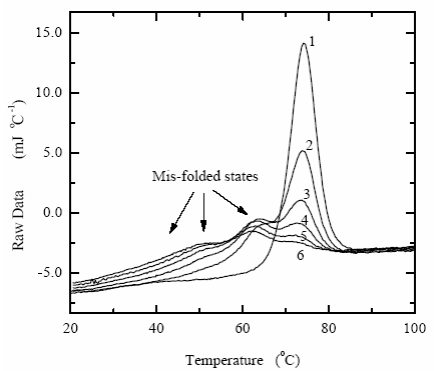
„ideal case“



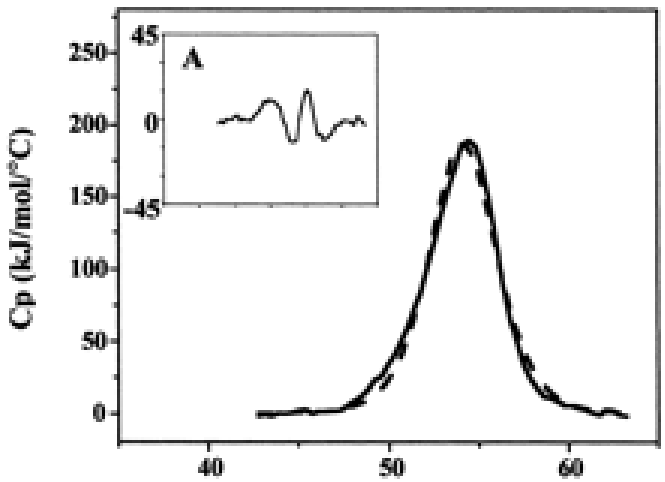

„real case“



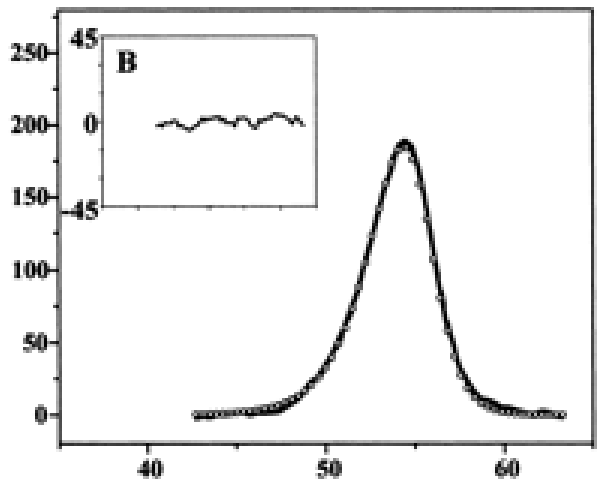




2-state model: F⇄U

# DSC of NAD$^+$ synthetase unfolding




$D_f \rightleftarrows 2U$




$D_f \rightleftarrows 2M_f \rightleftarrows 2U$

# DSC of proteins: protein stability

**$T_m$ is temperature midpoint**
-Related to protein stability

**$\Delta C_p$ almost always positive**
- Denaturated protein has higher heat capacity
(hydration of the solvent exposed side chains)

**Denaturated proteins susceptible to**
-Proteolysis
- Oxidation
- Deamidation
- Aggregation

**Factors responsible for folding and stability**
-pH, salt, temperature, H-bonding
non-covalent interactions, conformational entropy

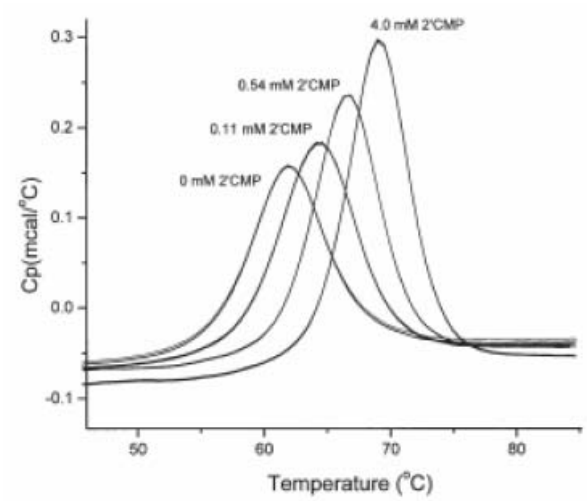
# DSC of ligand binding

**If ligand binds preferentially**
- Protein unfolding less favourable as [L] increases
- New $T_m$ occurs at higher temperature than in the absence of ligand ($T_o$)

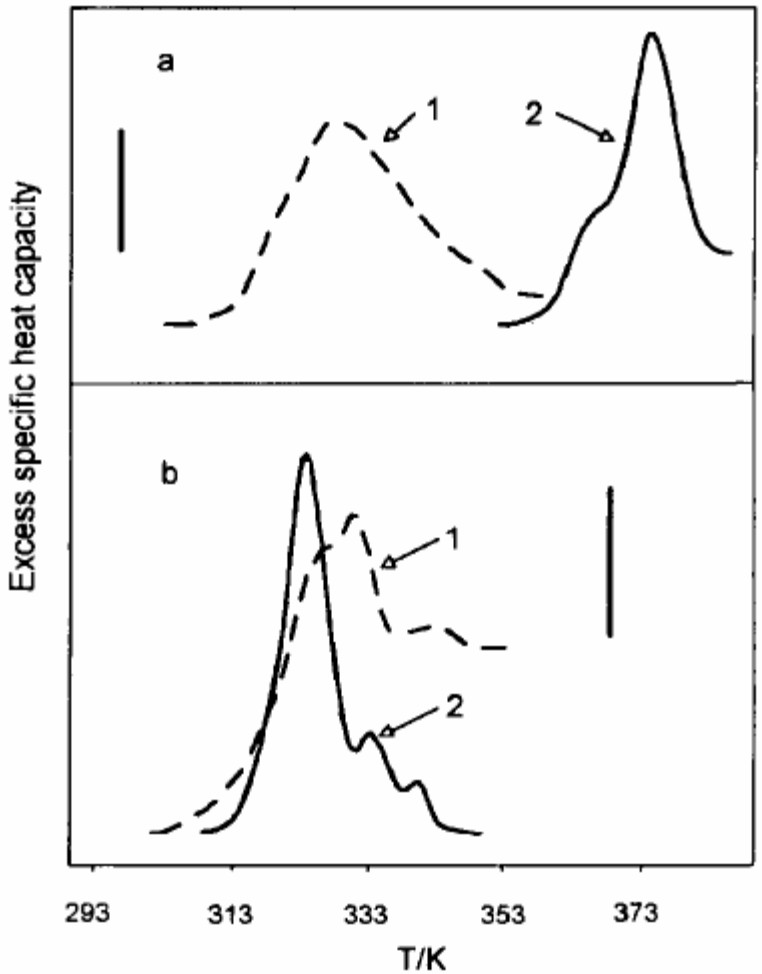
**Binding constant ($K_B$) may be estimated from**
- $T_m$, $T_o$, $\Delta H_{cal}$ and [L]

**Used to study protein interactions with**
-small ligands – drugs, inhibitors, peptides

**RNase A + 2´CMP**



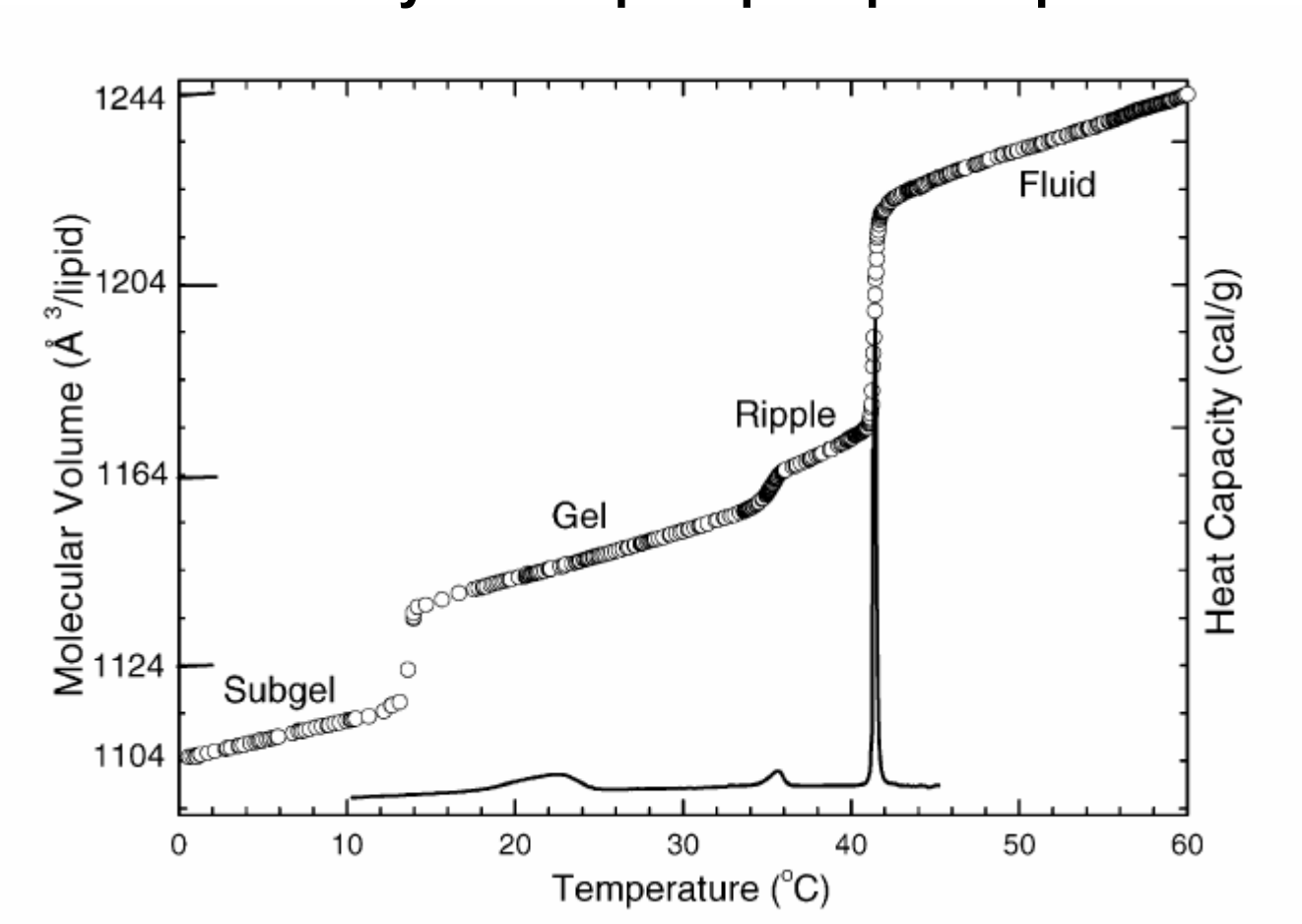
# DSC of Troponin complex interaction with $Ca^{2+}$



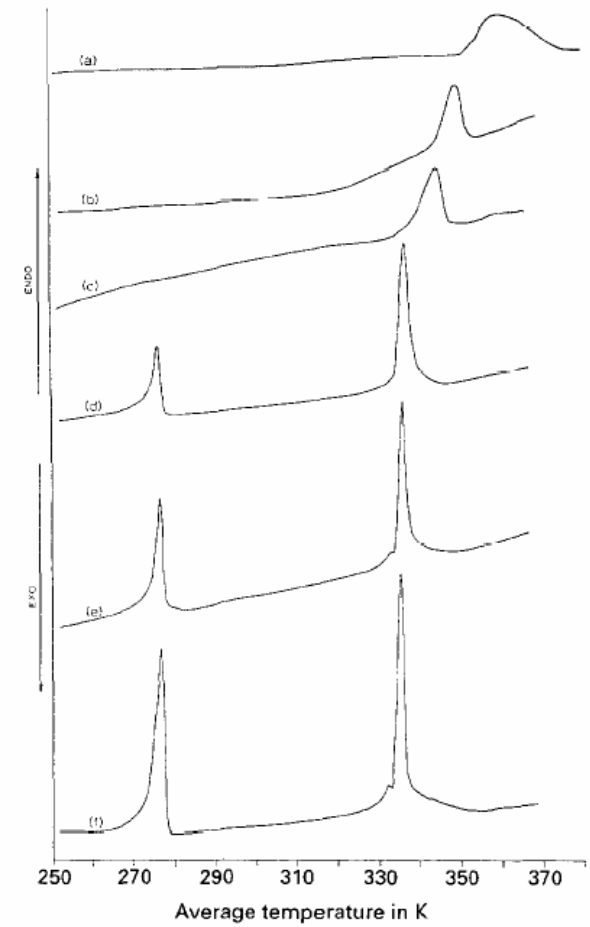

**Troponin C:**
1 – no $CaCl_2$
2 – added $CaCl_2$

**Complex of Troponins C/T/I:**
1 – no $CaCl_2$
2 – added $CaCl_2$

# DSC of synthetic phospholipid dispersion



# DSC of DSPC: hydration




Anhydrous

10% w/v $H_2O$

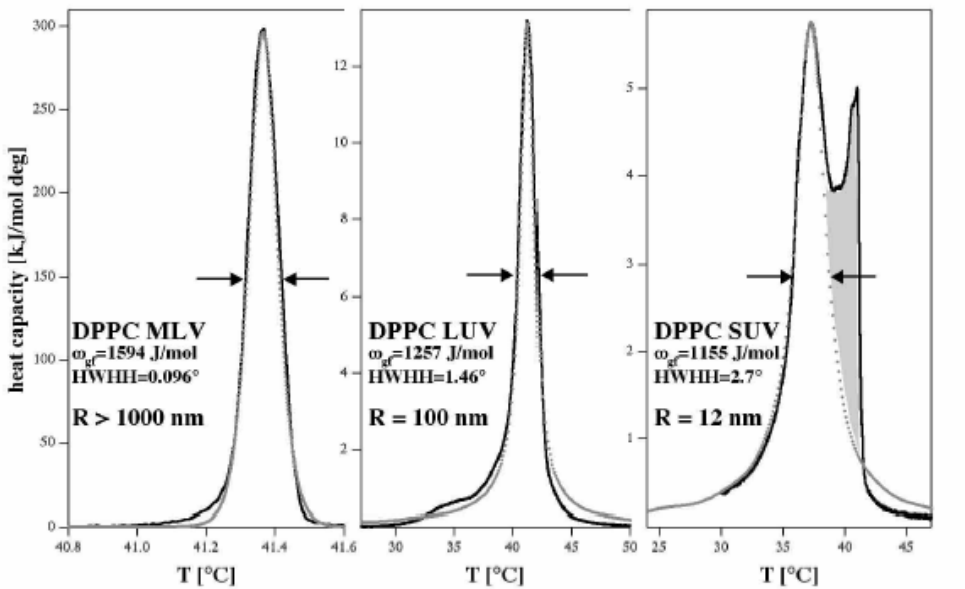
20% w/v $H_2O$

25% w/v $H_2O$

30% w/v $H_2O$

40% w/v $H_2O$

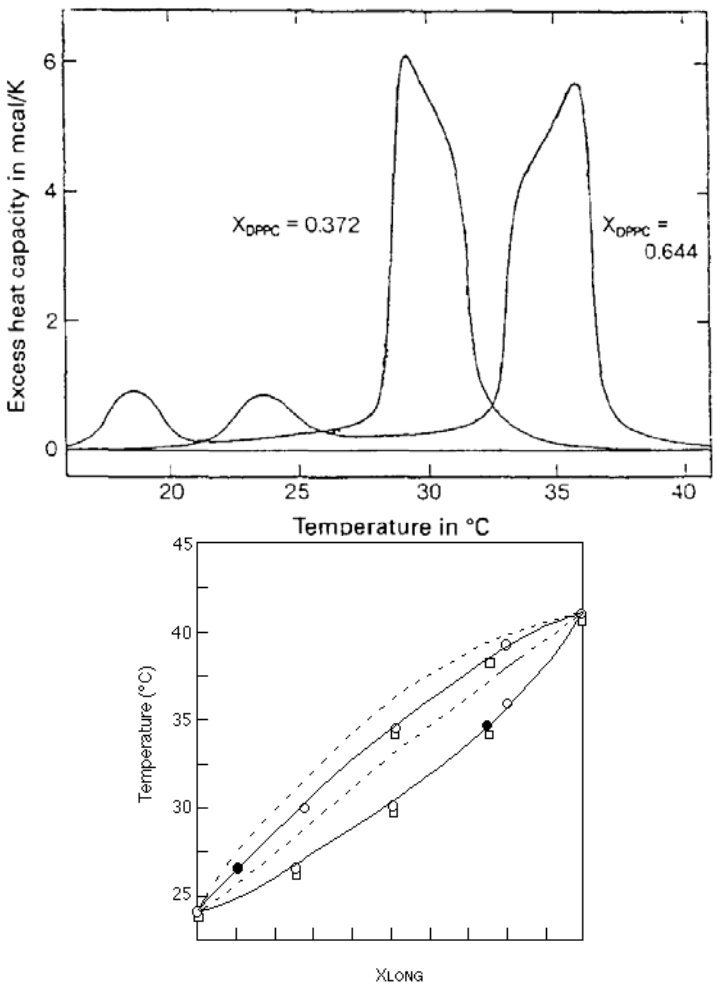
# DSC of phospholipids: lyposomes



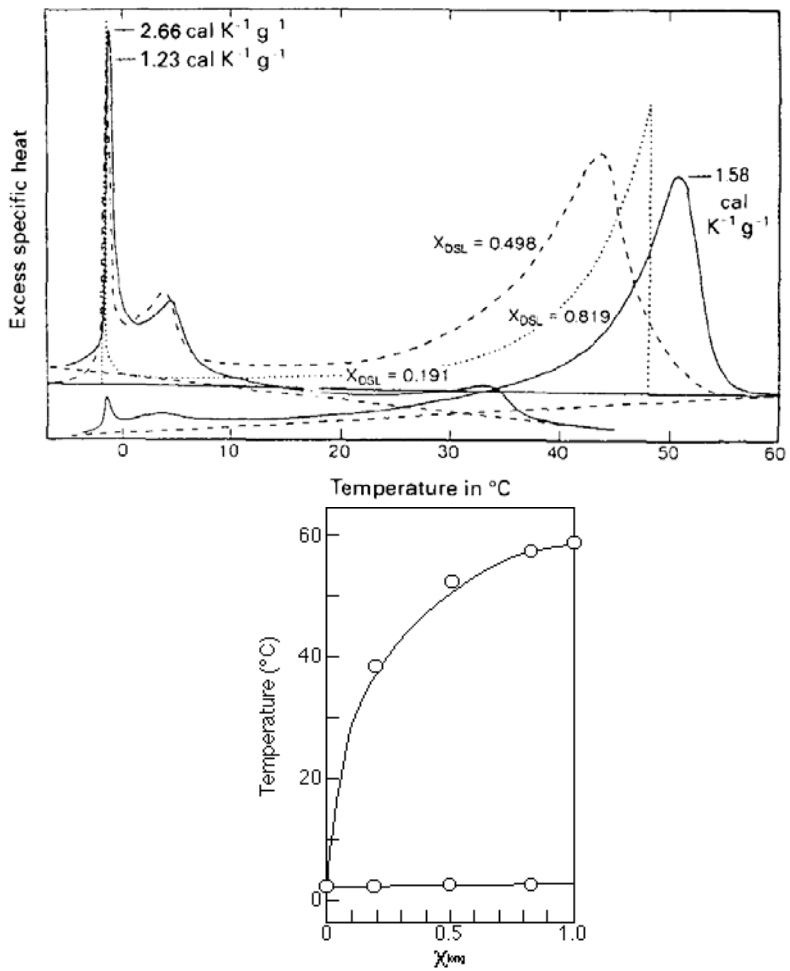

| | DMPC | | DPPC | |
|---|---|---|---|---|
| | Unsonicated | Sonicated | Unsonicated | Sonicated |
| Transition temperature/°C | 23 | 12 | 41 | 32 |
| Transition enthalpy/kcal $mol^{-1}$ | 6.6 | 3.3 | 8.6 | 6.0 |
| Transition entropy/cal $mol^{-1}$ $°C^{-1}$ | 22 | 11 | 28 | 19 |

# DSC of phospholipids: mixtures, phase diagramms

DPPC/DMPC

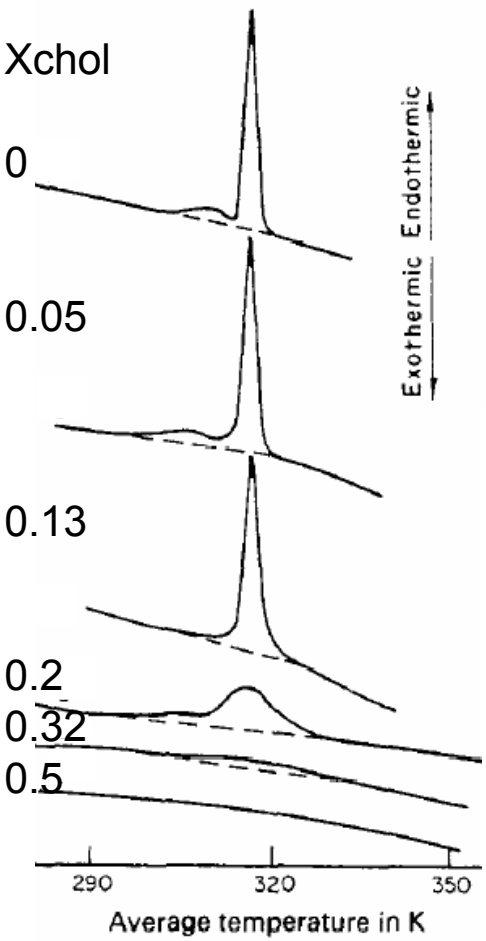


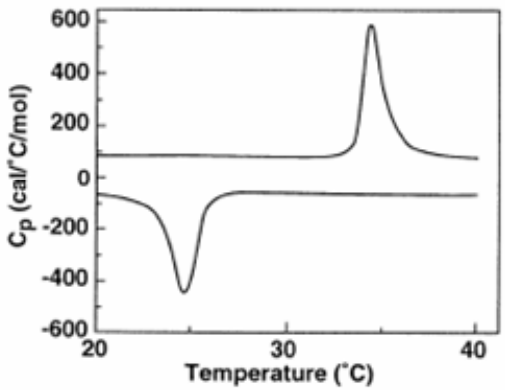

DLPC/DSPC



# DSC of phospholipids: cholesterol

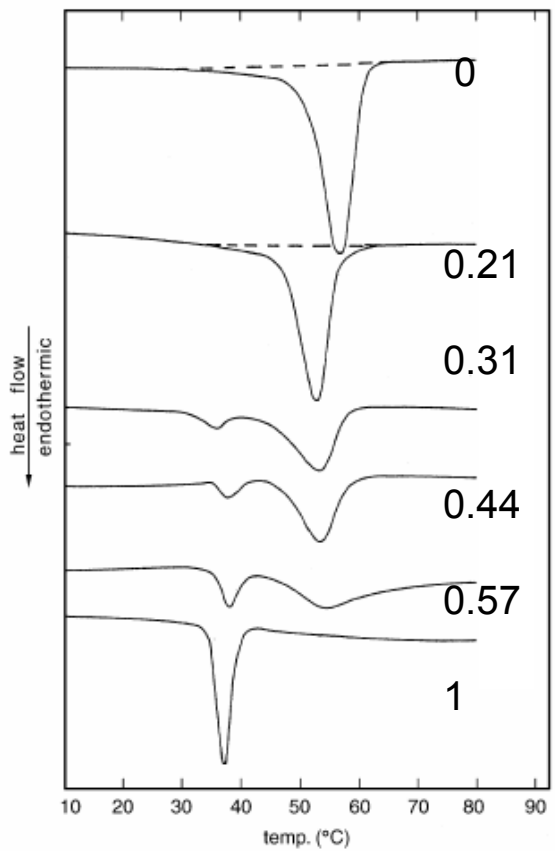
**DPPC**




DSC of cholesterol

**DPPS**



# DSC of phospholipids: cholesterol

Onset of phase separation of cholesterol from phospholipid/cholesterol mixtures

| Lipid | Charge | Presence of hydrogen bonds | Hydrocarbon chain length | Number of double bonds/hydro-carbon chain | Onset of cholesterol phase separation X(chol)[a] | |
|---|---|---|---|---|---|---|
| | | | | | Gel phase | l.c. phase |
| DMPC | (±) | no | 14 | 0 | | 0.45<br>0.5 |
| DPPC | (±) | no | 16 | 0 | 0.5 | |
| DSPC | (±) | no | 18 | 0 | >0.5 | >0.5 |
| SAPC | (±) | no | 18,20 | 0,4 | | 0.5 |
| DAPC | (±) | no | 20 | 4 | | 0.17 |
| Egg PC[b] | (±) | no | variable | variable | | 0.45–0.55 |
| DDPC | (±) | no | 22 | 6 | | 0.10 |
| DMPE | (±) | yes | 14 | 0 | 0.43 | 0.43 |
| DEPE | (±) | yes | 18 | 1[c] | | 0.35–0.4 |
| DOPE | (±) | yes | 18 | 1 | | 0.33[d] |
| Egg PE[b] | (±) | yes | variable | variable | | 0.35–0.4 |
| DMPG | (−) | no | 14 | 0 | 0.4 | 0.44 |
| DPPG | (−) | no | 16 | 0 | 0.38 | 0.37 |
| DSPG | (−) | no | 18 | 0 | 0.32 | 0.35 |
| POPG | (−) | no | 16,18 | 0,1 | | 0.45 |
| DMPS | (−) | yes | 14 | 0 | 0.33 | 0.37 |
| DPPS | (−) | yes | 16 | 0 | 0.3 | |
| POPS | (−) | yes | 16,18 | 0,1 | | 0.36 |
| SOPS | (−) | yes | 16,18 | 0,1 | | 0.2 |
| PS[b,e] | (−) | yes | variable | variable | | 0.3 |

# DSC of phospholipids with small molecules (peptides?)

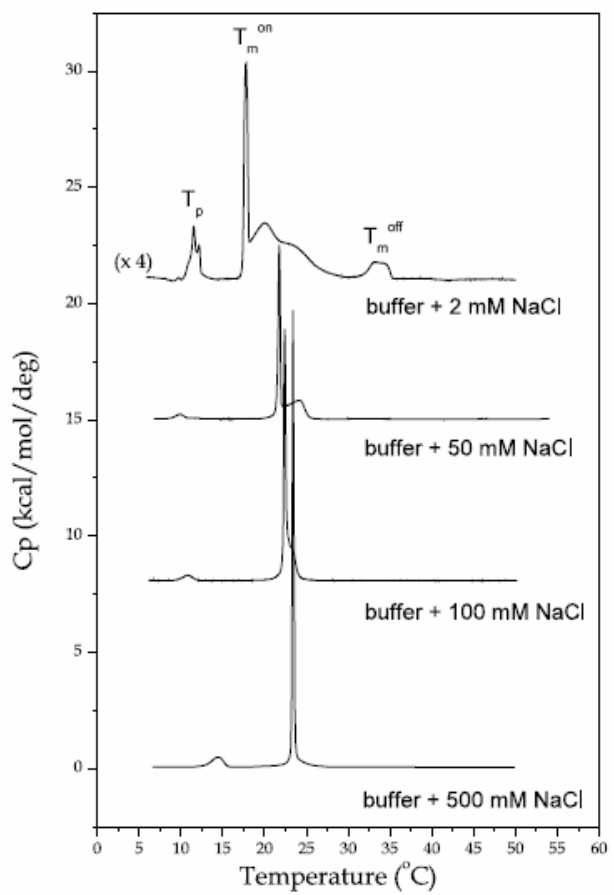
**Type A:** down shift of $T_m$, increase in $T_{1/2}$ constant $\Delta H_{cal}$
*Partually buried in the bilayer, interact with $C_2$-$C_8$ methylens*

**Type B:** appear of the shoulder, that grows into new peak with [additive], constant $\Delta H_{cal}$ of both peaks
*Located on the interface, interact with glycerol backbone*

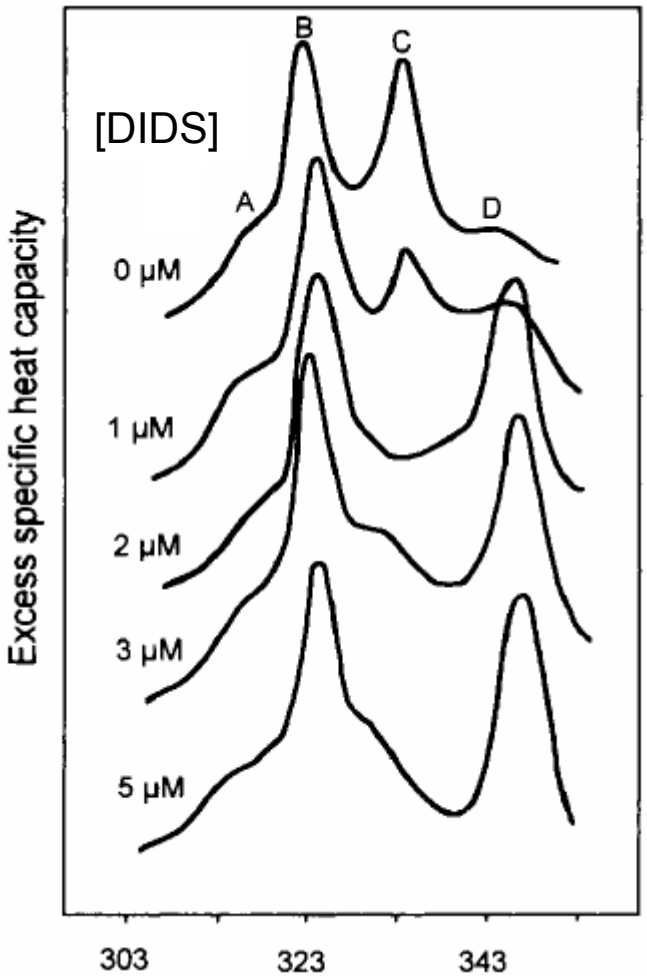
**Type C:** down shift of $T_m$, constant $T_{1/2}$ , $\Delta H_{cal}$
*Located in the center of bilayer, interact with $C_9$-$C_{16}$ methylens*

**Type D:** discrete new peak that grows in expence of the previous
*Located on the surface, interact with the headgroups*

# DSC of phospholipids: DMPG



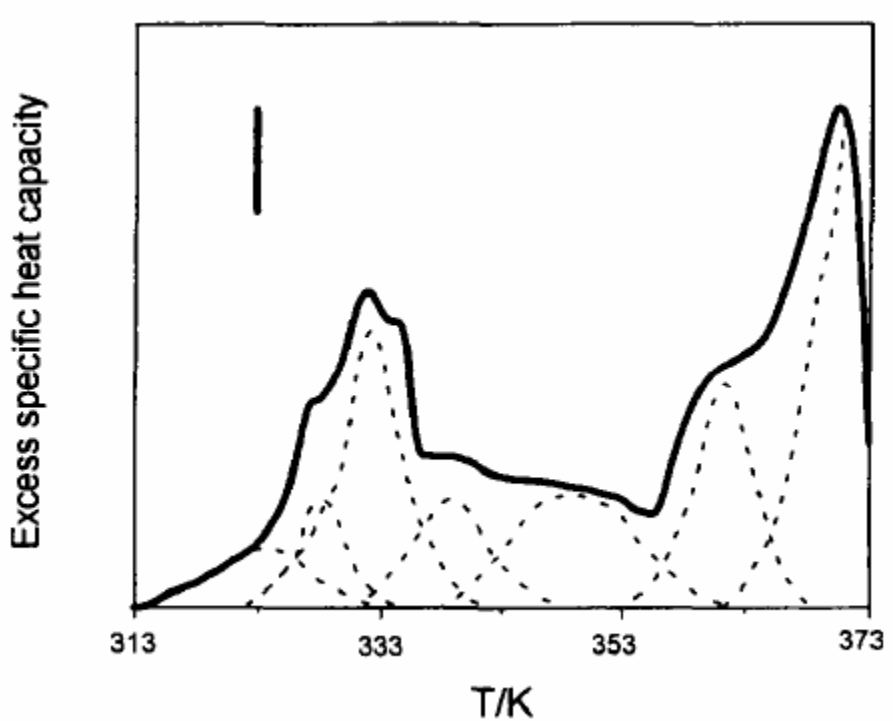
# DSC of a biological membrane




Erytrocyte ghosts:

A: Spectrin
B: Extramembrane domains
C,D: Band 3

# DSC of a cell




Cock sperm suspension

# Thanks!