Lecture 17:
Denaturation of Globular Proteins & Cooperative Transitions

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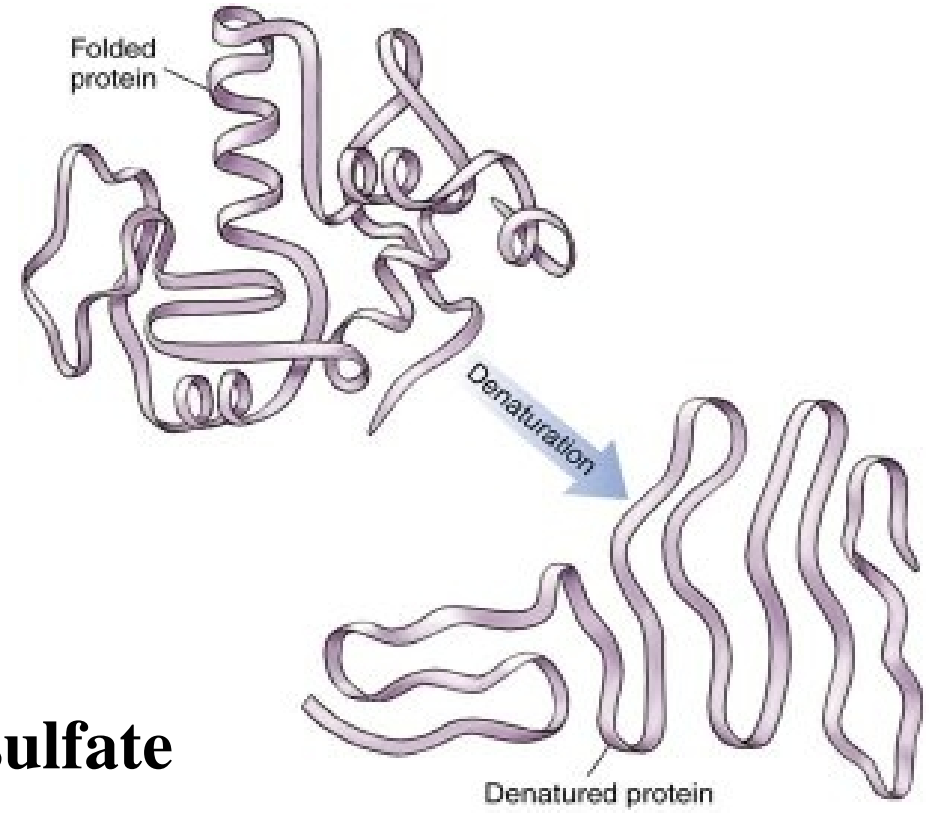
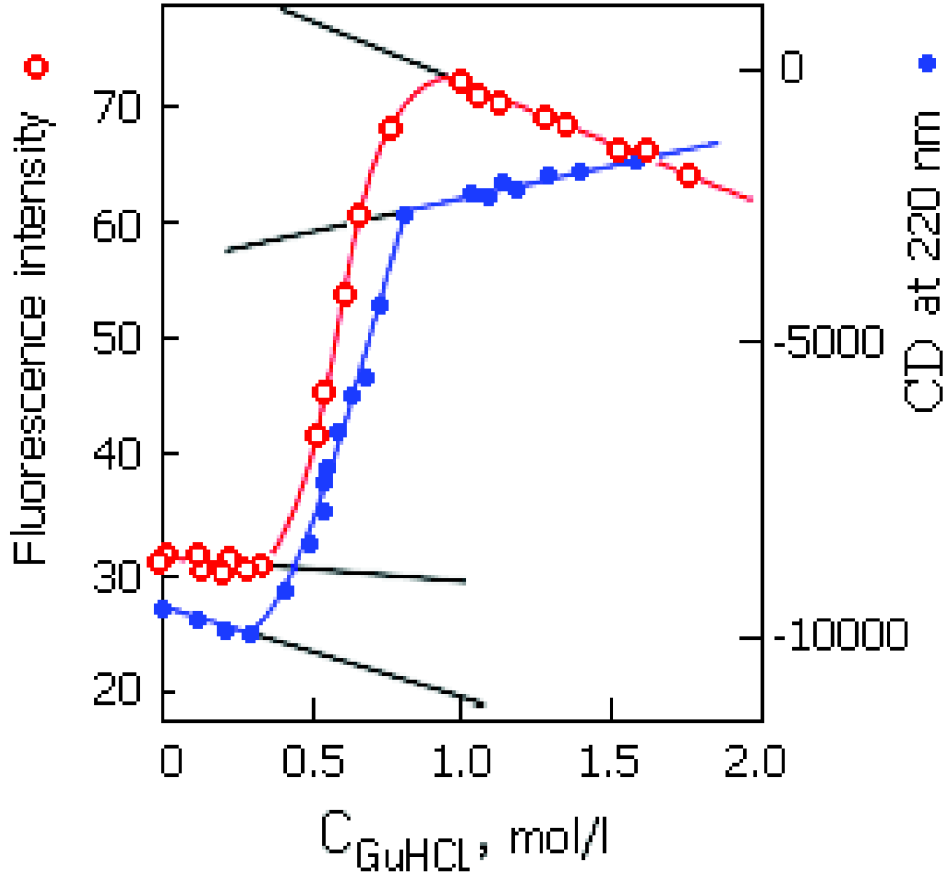
Protein Denaturation ⇔ Loss of the Native 3D Structure

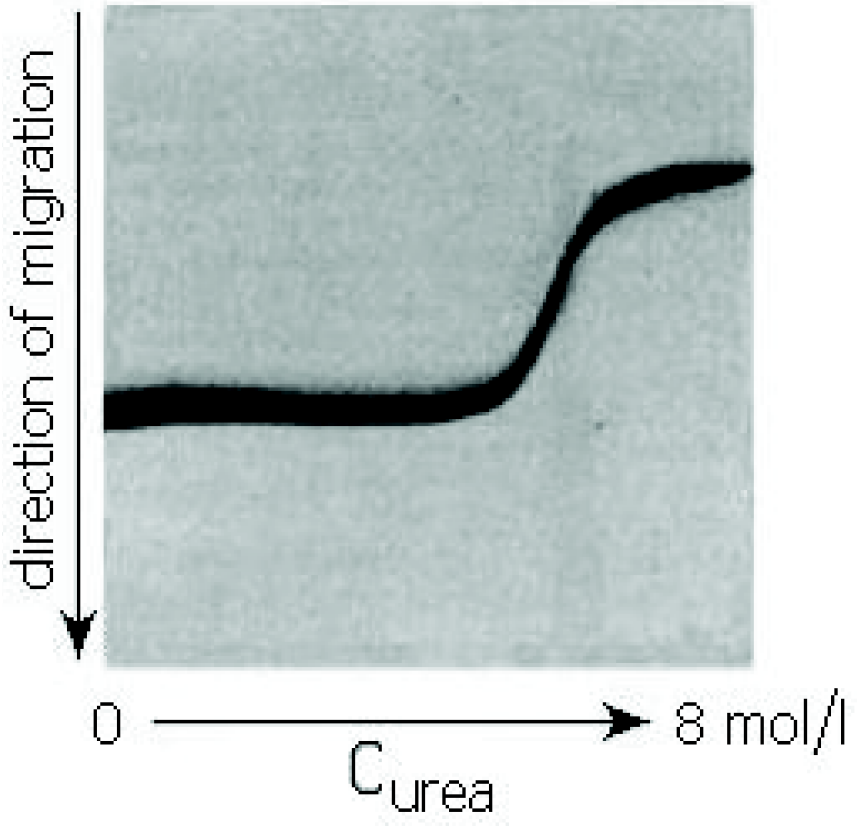
- ***in vitro*** **denaturation: - abnormal temperature**
  - abnormal pH (H^+ or OH^- ions)
  - denaturants (e.g. urea, SDS*)
- ***in vivo*** **(in the cell) denaturation**
- **the topic of this lecture: denaturation of water-soluble proteins**



SDS* – sodium dodecyl sulfate

Protein Denaturation: S-shaped Changes in Some Quantities
- fluorescence & CD intensities (left)
- migration during electrophoresis (right)



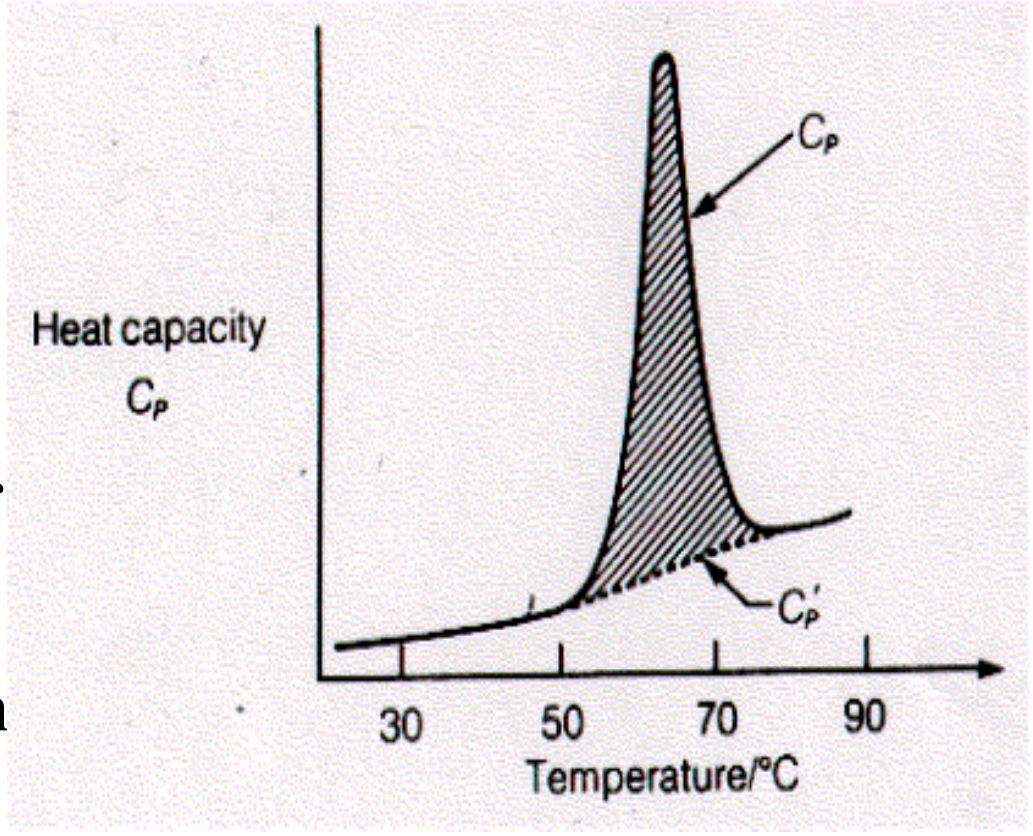


Calorimetric Studies: Heat Capacity of Denaturation

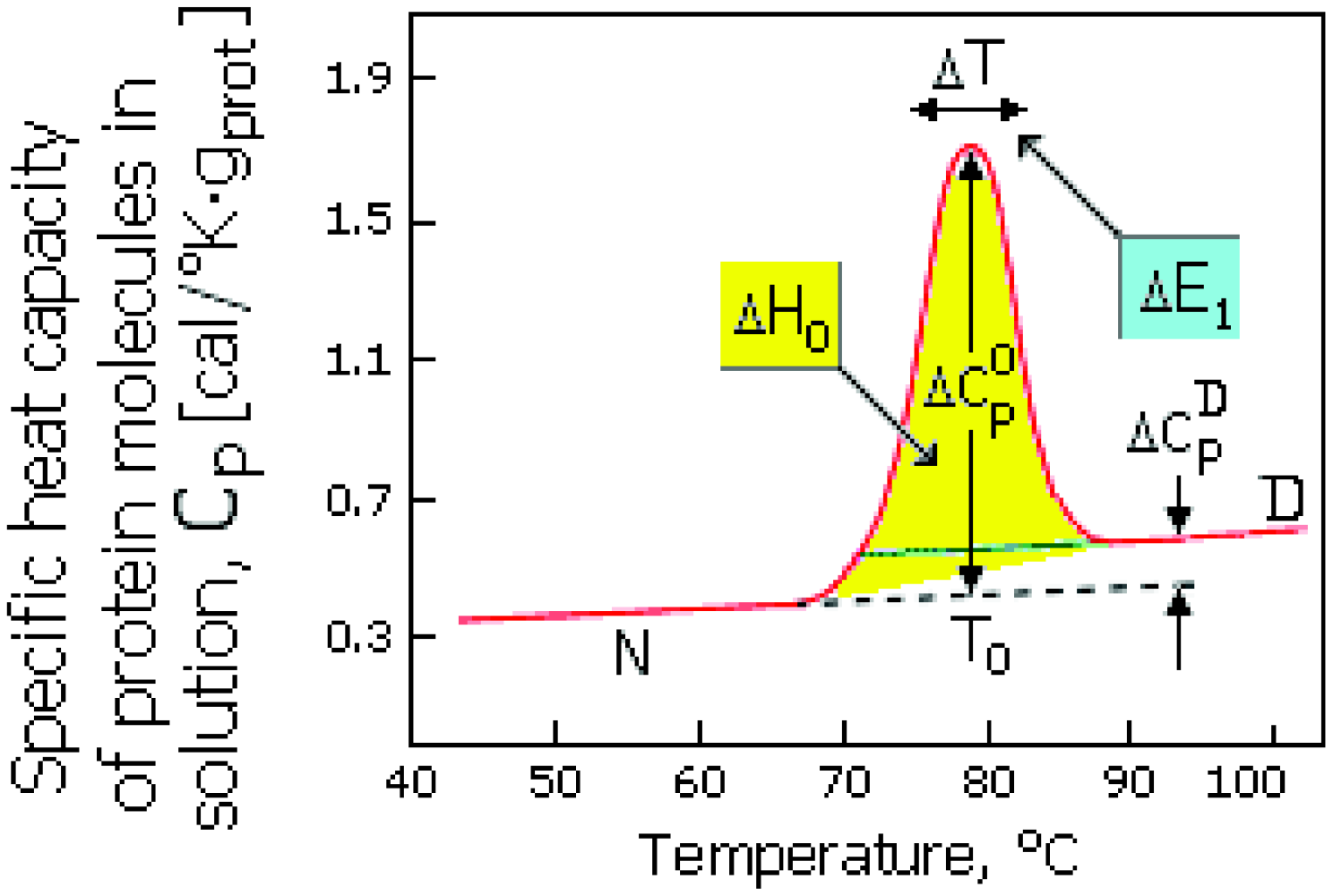
- **the shaded area: the enthalpy (ΔH) absorbed during the denaturation: $\Delta H = C_P \Delta T$**

- **ΔH used to break the non-covalent bonds in the globular protein**

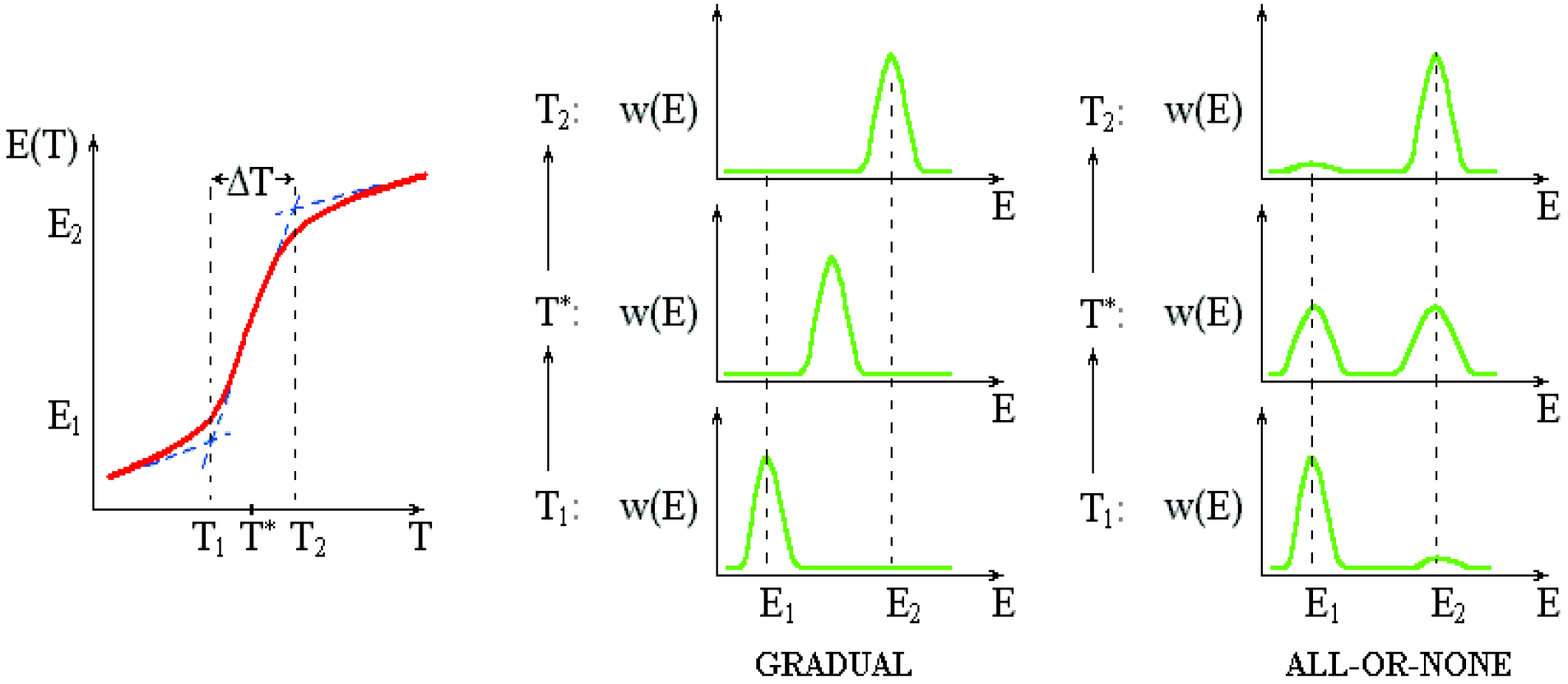
- **$C_P' \Delta T$– contribution of the solvent (expanding the buffer volume durin temperature increase)**



ΔT – non-zero width of the denaturation transition



Is Protein Denaturation All-Or-None OR Gradual Transition? How can we tell? What is the criterion?



Van't Hoff's Criterion for existence of "all-or-none" Transition:

- Comparison of
  - the "effective heat" of transition, ΔE,
    (the heat consumed by one "melting unit")

  - the "calorimetric heat" of the transition, $\Delta H/N$,
    (the heat per one melting protein molecule,
    N – number of protein molecules)

- van't Hoff's criterion: if $\Delta E = \Delta H/N$, transition is ALL-OR-NONE

If $\Delta E < \Delta H/N$, the "melting unit" is smaller than one protein molecule ⇨ the protein melts in parts.

The effective heat of the transition is related to the width ΔT:

- **"melting unit" can be in 2 states:**
  - **(1) with energy E and entropy S (solid or native)**
  - **(2) with energy E' and entropy S' (molten)**
- **assume: E, S, E', S' do not depend on T**
- **according to Boltzmann statistics, probability to be in the molten state P_{MOLTEN}: (ΔE=E'-E & ΔS = S'-S)**

$$P_{MOLTEN} = \exp[-(E'-TS')k_B T]/$$

$$\{\exp[-(E'-TS')k_B T]+\exp[-(E-TS)k_B T]\}$$

$$= \{1+\exp[(\Delta E-T\Delta S)/]k_B T\}^{-1}$$

$$P_{SOLID} = 1 - P_{MOLTEN}$$

How sharp is the transition?

– $dP_{MOLTEN}/dT = ?$

– for an easier derivation: $X = (\Delta E - T\Delta S)/k_B T$

$$P_{MOLTEN} = (1 + e^X)^{-1}$$

$$P_{SOLID} = e^X (1 + e^X)^{-1}$$

$$– dP_{MOLTEN}/dT = dP_{MOLTEN}/dX \times dX/dT$$

$$= – e^X (1 + e^X)^{-2} \times [– (\Delta E – T\Delta S)/k_B T^2 – \Delta S/k_B T]$$

$$\Downarrow$$

$$dP_{MOLTEN}/dT = P_{MOLTEN}(1 – P_{MOLTEN}) \Delta E/k_B T^2$$

– the mid-transition: $T_0 = \Delta E/\Delta S$ & $P_{MOLTEN}(T_0) = P_{SOLID}(T_0) = ½$

– the maximum of dP_{MOLTEN}/dT is close to T_0 (if $\Delta E/k_B T \gg 1$)

$$(dP_{MOLTEN}/dT)_{T=T0} = ¼\, \Delta E/k_B T_0^2$$

– in the region ΔT: P_{MOLTEN} changes $0 \rightarrow 1$

– $(dP_{MOLTEN}/dT)_{T=T0} = \Delta P_{MOLTEN}/\Delta T = 1/\Delta T$

$$\Downarrow$$

$$1/\Delta T = ¼\, \Delta E/k_B T_0^2$$

$$\Delta E = 4k_B T_0^2 / \Delta T$$

– melting unit ΔE calculated from the S-shape of the transition

– heat absorbed by one protein molecule: $\Delta E_1 = \Delta H/N$

(N = m/M – number of protein molecules

m – total mass & M – molecular mass of the protein)

Three possible outcomes:

(1) $\Delta E < \Delta H/N$ – "melting unit" smaller than one protein molecule (protein melts in parts)

(2) $\Delta E = \Delta H/N$ – "melting unit" exactly one protein molecule (all-or-none transition)

van't Hoff criterion

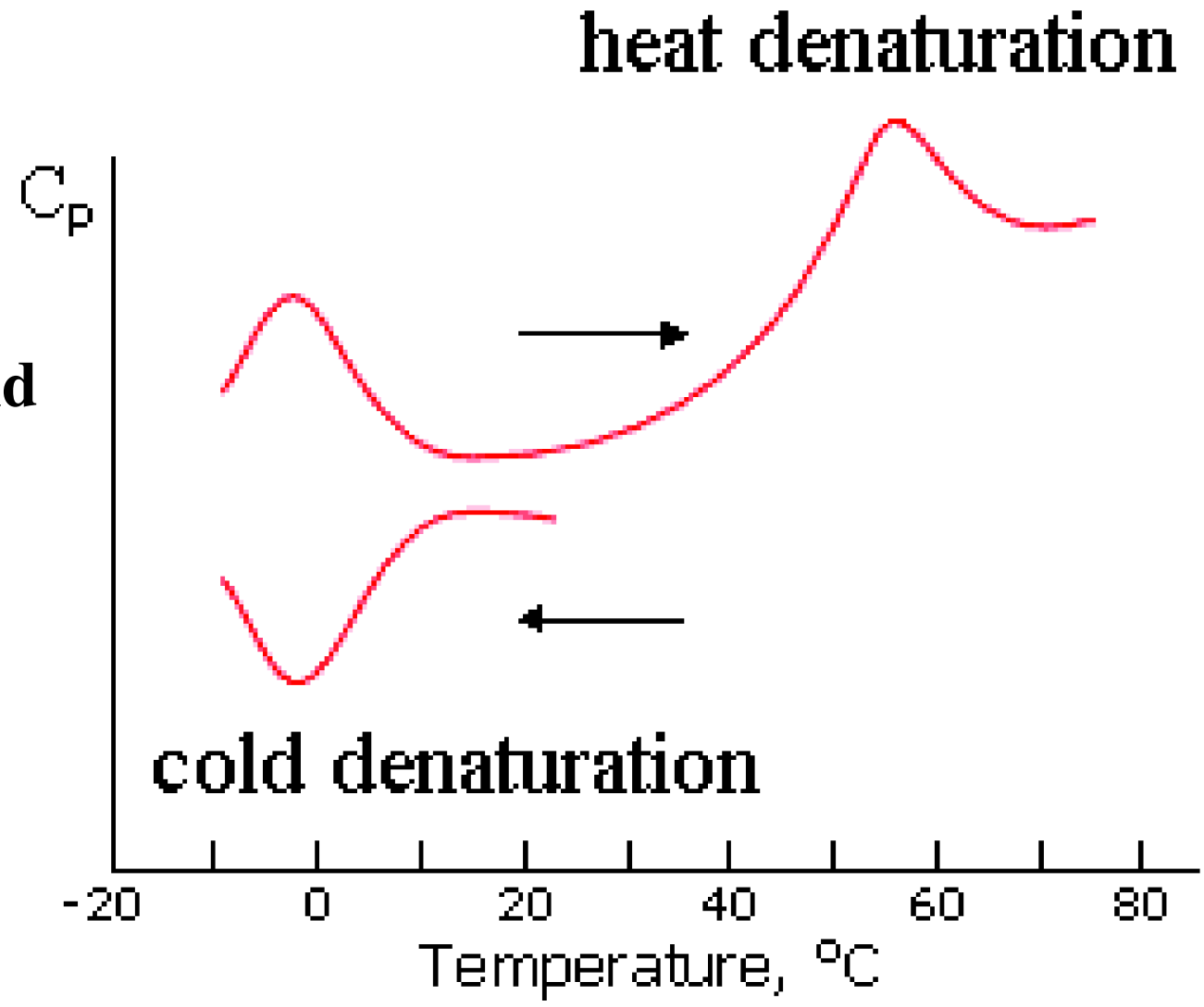
(3) $\Delta E > \Delta H/N$ – "melting unit" larger than one protein mol. (melting of an aggregate)

Protein Melting – A Result of an Elevated Temperature

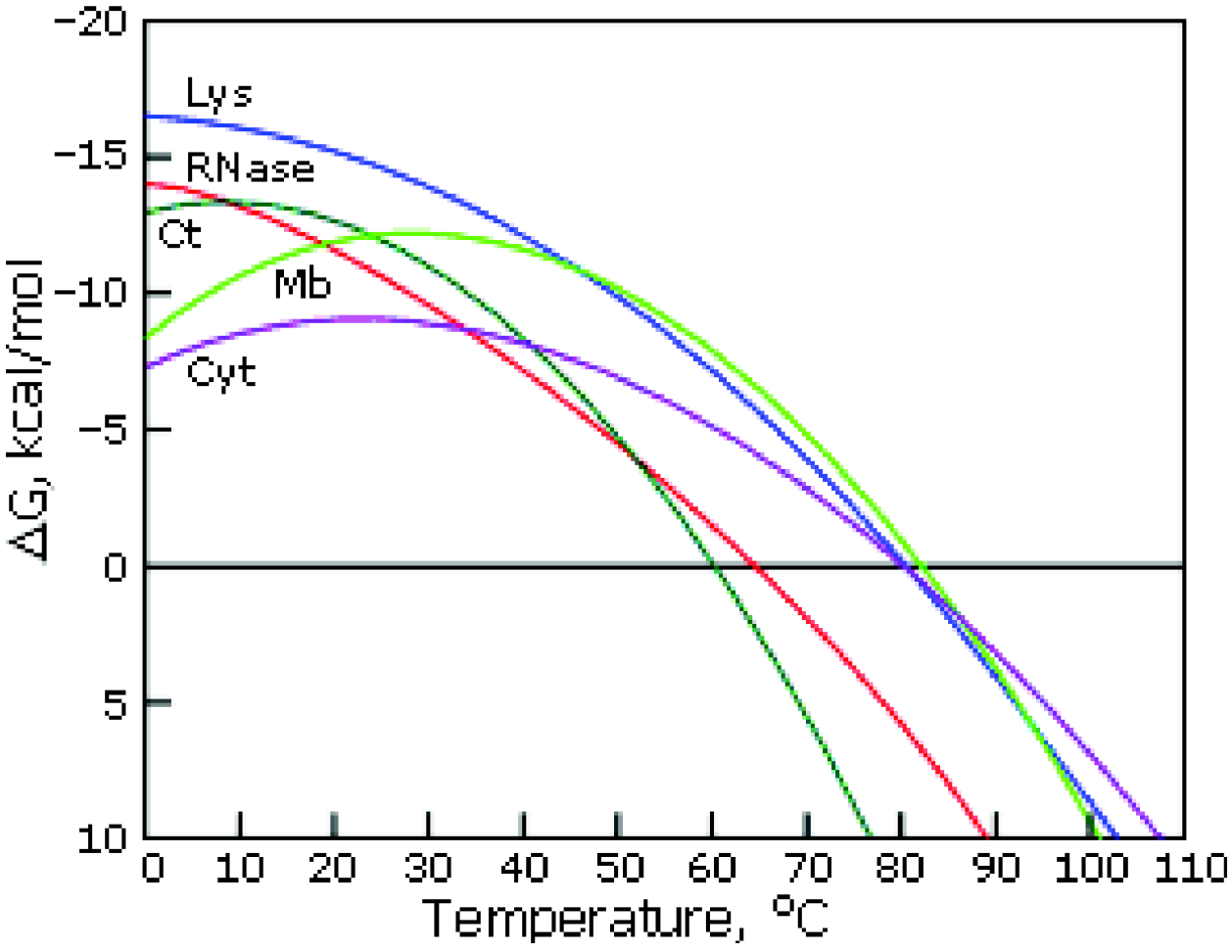
Decay of Protein Structure Observed Also at Abnormally Low Temperatures ⇨ COLD DENATURATION

Experimental Evidence for Cold Denaturation

both heat & cold denaturation "all-or-none" transitions !



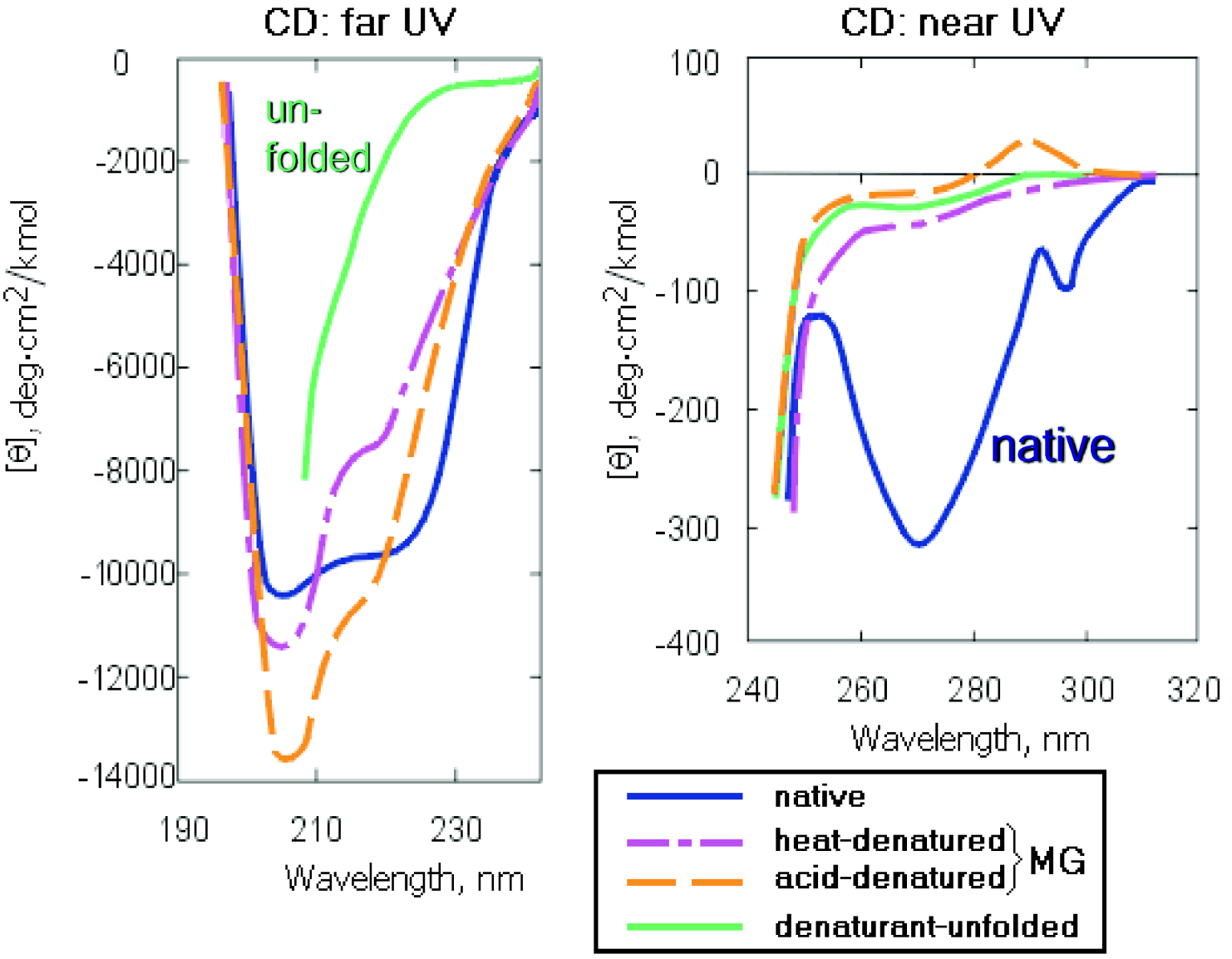
- strength of hydrophobic/hydrophilic effect increases with T
- at temperatures below ~10°C, hydrophobic forces WEAK
- the native structure is the most stable at room Ts
- protein melting at T ~ 60-80°C
- different curves for different proteins
- for large number of proteins, T of denaturation < freezing T of water



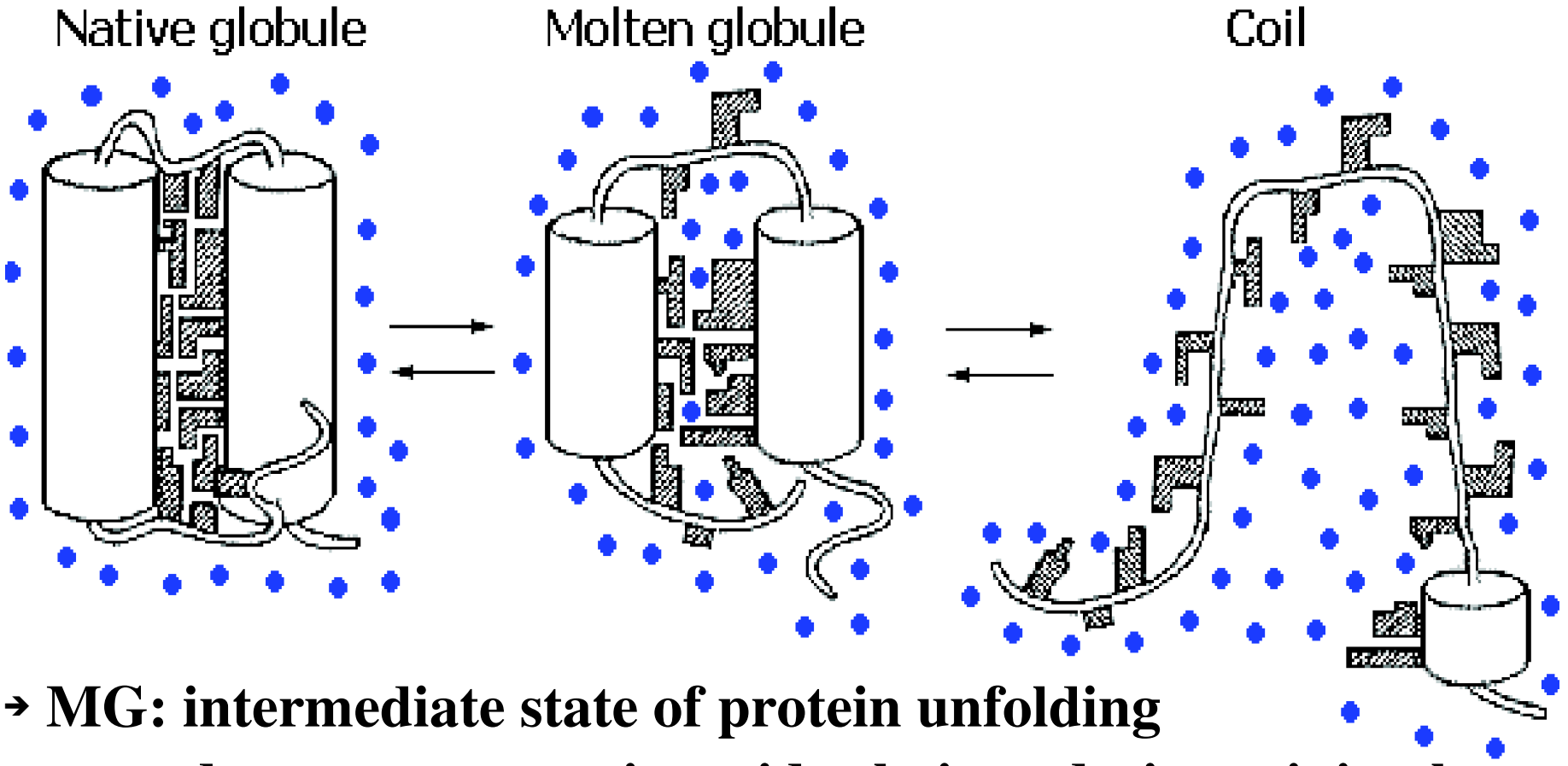
How does a denatured protein look like? Experimental data

-far UV (secondary structure)

-near UV (aromatic side chains)



Consensus: ∃ of the molten globule (MG) state



- MG: intermediate state of protein unfolding
- secondary structure exists, side chain ordering minimal
- some native contacts (involving aromatic chains, NMR data)
- solvent easily penetrates into the MG state

MAIN PROPERTIES OF MOLTEN GLOBULE STATE
and methods of their registration

"GLOBULE" (like native protein)		"MOLTEN" (unlike native protein)	
Hydrodynamic volume, Small- and medium-angle X-ray scattering	⇔ COMPACTNESS	Near UV CD, H^1 NMR spectra	⇔ NO UNIQUE PACKING OF SIDE CHAINS
Large-angle X-ray scattering	⇔ PRESENCE OF CORE	H↔D exchange + NMR, Proteolysis	⇔ FLUCTUATIONS
NMR (spin echo)	⇔ SOME AROMATIC SIDE CHAINS ARE FIXED	NMR (spin echo)	⇔ MOBILITY OF ALIPHATIC SIDE CHAINS
Far UV CD, IR spectra, NMR + H↔D exchange	⇔ SECONDARY STRUCTURE	Scanning & isothermal microcalorimetry	⇔ NO FURTHER MELTING (as a rule)
Fluorescence	⇔ SOME Trp RESIDUES ARE NOT ACCESSIBLE TO WATER	Fluorescence	⇔ SOME Trp RESIDUES ARE ACCESSIBLE TO WATER
2D NMR	⇔ PRESERVATION OF SOME LONG-RANGE CONTACTS	2D NMR	⇔ DECAY OF MOST LONG-RANGE CONTACTS
Chromatography (HPLC)	⇔ POSSIBILITY OF "CORRECT" S-S BOND FORMATION		

DIFFERENCE from both native and unfolded states
Enhanced binding of non-polar molecules
(fluorescence of protein-bound hydrophobic dye, ANS)

COURSE EVALUATION INSTRUCTIONS:

(1) Your course evaluation responses help us improve teaching and are greatly appreciated. They are anonymous and you are free to participate or not.

(2) The lecture today will finish 15 minutes earlier to allow you to do the course evaluation on-line.

(3) On-line evaluation can be done on the workstations available in the room 12-704 (or use your laptops or other workstations you can access). Login on the workstations in 12-704 via
Login name: student_survey
Password: physics_rules!
in the case that you already do not have your own account.

(4) Course evaluation is set as an assignment on your BbVista home page. Login there to fill out the survey.