

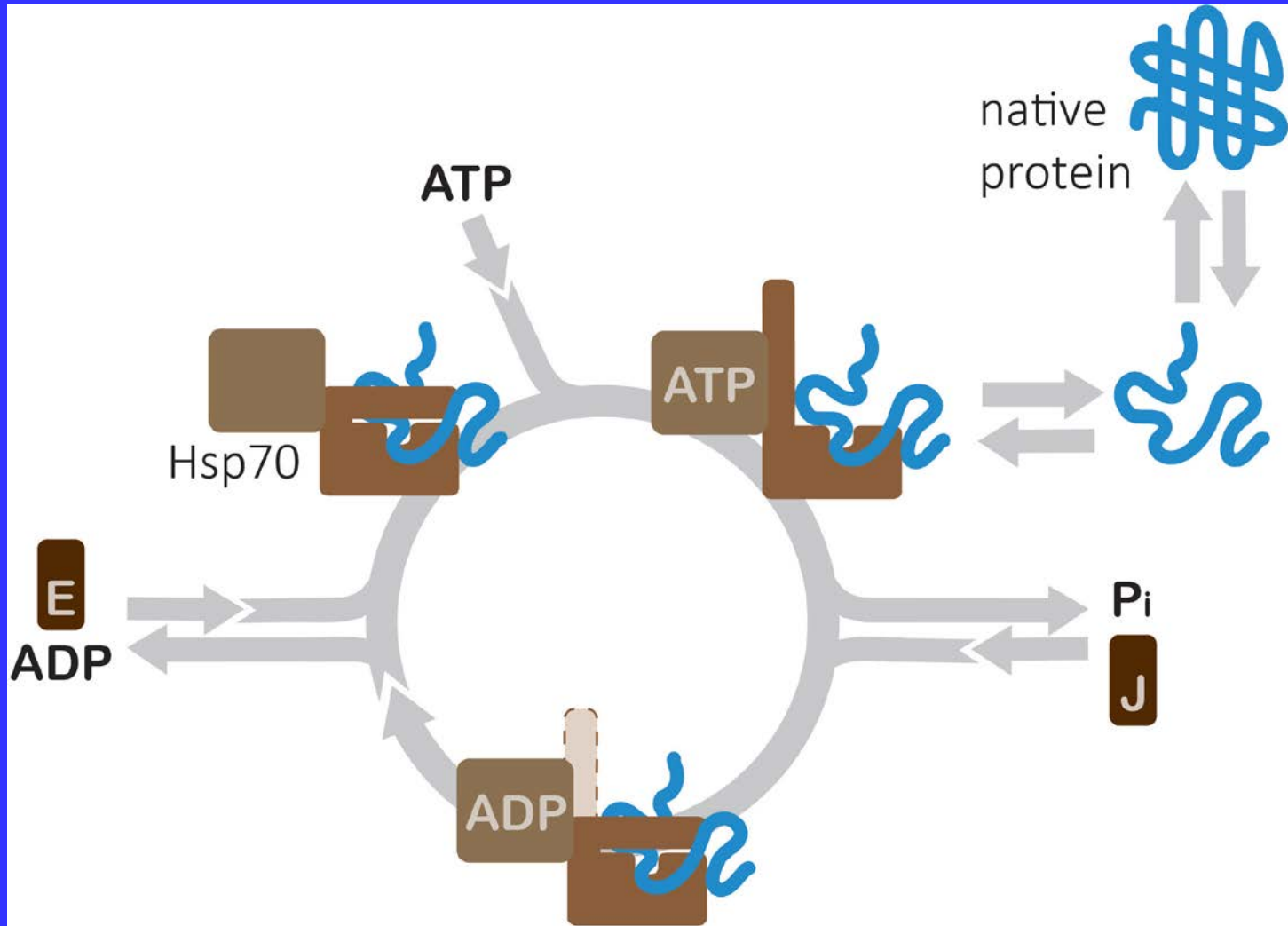
PRACTICAL COURSE IN CELL BIOLOGY (LVA 941.337)

- Start: Monday, 9:00
- Finish: Friday, ≈16:00 (last microscopy group)
- !! Please bring lab coats, indoor shoes and USB sticks !!
- Evaluation: report (1 x per group of 2); questionnaire (→ course manual): fill in by hand!

Heat Shock Protein 70 (hsp70)

- Synthesis induced by cellular stress
- has a molecular mass of 70 kDa
- acts as molecular chaperone → contributes to folding of cellular proteins
- binds to newly-synthesized proteins
- prevents their aggregation
- this process requires energy
 - ATP hydrolysis

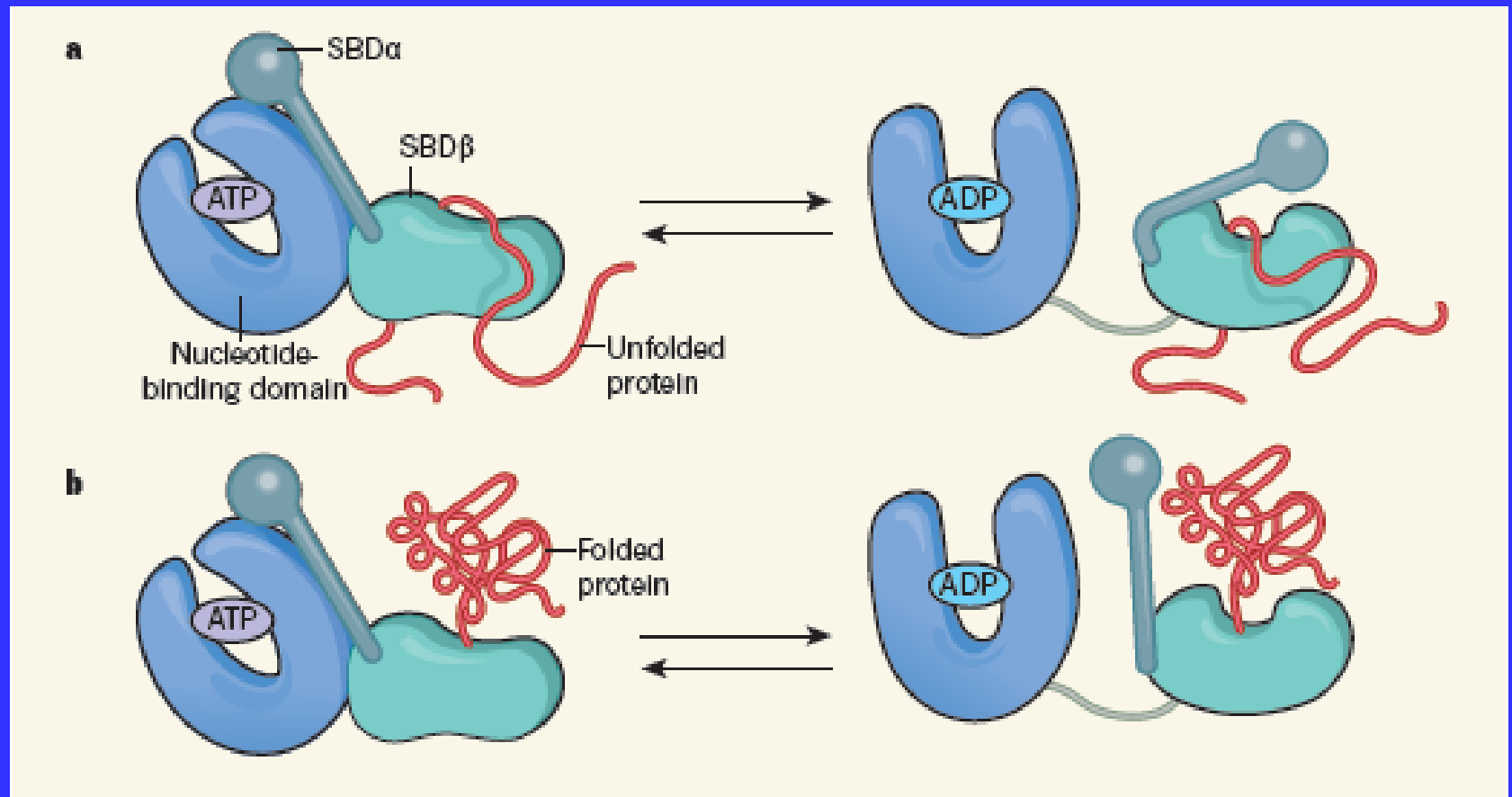
The hsp70 reaction cycle



Structure and function of hsp70

- consists of ATPase and substrate-binding domains
- latent ATPase activity causes hydrolysis of ATP into ADP
- hsp70 then traps incorrectly folded proteins
- ADP dissociation permits again binding of ATP
- ATP-induced conformational change triggers release of bound proteins
- hsp70 does also interact with selected folded proteins

Hsp70 interactions with proteins



Course experiments

- Induction of hsp70 by heavy metals (cadmium chloride)
- Detection of hsp70 protein (immunoblotting)
- Detection of hsp70 mRNA (RT-PCR)
- Detection of the activity of the hsp70 promoter with Green Fluorescent Protein (GFP) as reporter

Immunoblotting

- Separation of cellular proteins by SDS-PAGE
- Transfer of separated proteins onto a nitrocellulose membrane → Western Blotting
- Labelling of hsp70 with a specific antibody
- Detection with a secondary antibody coupled to horseradish peroxidase (HRP)
- HRP oxidises luminol → chemiluminescence
- Loading control: GAPDH

Reverse transcriptase-PCR (RT-PCR)

- Isolation of cellular RNA
- Reverse transcription (RNA → cDNA)
- Polymerase chain reaction (PCR)

analysed cDNAs: hsp70

GAPDH (control)

- GAPDH is expressed constitutively

Promoter analysis

- hsp70 promoter drives expression of GFP
- Construct stably expressed in LTK transfectants → LHG8 cells
- Treatment of cells with cadmium chloride → induction of GFP synthesis
- GFP production visualized by fluorescence microscopy