Review

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The emerging role of ATP as a cosolute for biomolecular processes

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Abstract: ATP is an important small molecule that appears at outstandingly high concentration within the cellular medium. Apart from its use as a source of energy and a metabolite, there is increasing evidence for important functions as a cosolute for biomolecular processes. Owned to its solubilizing kosmotropic triphosphate and hydrophobic adenine moieties, ATP is a versatile cosolute that can interact with biomolecules in various ways. We here use three models to categorize these interactions and apply them to review recent studies. We focus on the impact of ATP on biomolecular solubility, folding stability and phase transitions. This leads us to possible implications and therapeutic interventions in neurodegenerative diseases.

Keywords: ATP; cosolute; phase transitions; protein aggregation; neurodegenerative diseases; hydrotrope

1 Molecular functions of ATP in the cell

For a long time, it has been known that adenosine triphosphate (ATP) is the central energy currency of the cell. It is produced from dietary supplies such as glucose, glycerol or fatty acids through glycolysis, the citric acid cycle and subsequent oxidative phosphorylation. The energy preserved in phosphorus-oxygen bonds can be made available by hydrolysis into either adenosine diphosphate (ADP) and inorganic phosphate (ΔG_0 (aqueous solution)=−30.5 kJ/mol; ΔG_0 (cytosol)=−57.0 kJ/mol (Nicholls 2003; Berg et al. 2007)) or adenosine monophosphate (AMP) and pyrophosphate (ΔG_0 (aqueous solution)=−45.6 kJ/mol (Berg et al. 2007)). Due to this high energy content, the most important task of ATP in the cell is to provide energy for a plethora of cellular processes such as contraction of muscles or the active transport of molecules. In this function ATP also supports the upkeep of cellular homeostasis by providing energy to protein and RNA chaperones such as Hsp90 (Panaretou et al. 1998), Hsp70/DnaK (McCarty et al. 1995) and various DEAD box proteins (Weis 2021). Further, ATP serves as a substrate to kinases which activate other molecules through phosphorylation. It is also required by DNA and RNA polymerases and by aminoacyl-tRNA synthetases to activate amino acids via adenylation prior to coupling the activated amino acid to its corresponding tRNA. Furthermore, ATP acts as a neurotransmitter in purinergic signaling. These are only few examples for the many roles and functions fulfilled by ATP.

ATP is present in living cells in an outstandingly high concentration in the millimolar range (2–10 mM) (Beis and Newsholme 1975; IT'IS Foundation 2022; Traut 1994). This is remarkable, particularly when compared to the much lower concentrations of other metabolites and small solutes. In fact, this concentration is 10–100 times higher than the Michaelis constant K_M of most enzymes showing ATPase activity (Rice and Rosen 2017). Hence, it is an intriguing question why cells deliberately maintain such high ATP concentration. At equilibrium, the ATP-ADP concentration ratio would be about 1:10,000,000 ($\triangleq\Delta G_0$=0 kJ/mol). In the cytoplasm, it is found to be rather 1000:1 ($\triangleq\Delta G_0$=−57 kJ/mol), thus out of equilibrium by several orders of magnitude (Nicholls 2003). ATP is quite stable kinetically and only decomposes into ADP and phosphate with a half-life of several years in the absence of an enzyme catalyzing this reaction (Stockbridge and Wolfenden 2009; Westheimer 1987; Wolfenden 2011). Still, building up and maintaining such a high ATP concentration against the concentration gradient is still laborious and would be unnecessary just to meet the requirements of the cell for ATP as a provider of energy. Thus, the question why cells build and maintain such a high cellular ATP concentration seems quite compelling.

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In a study published in 2017, Patel et al. (2017) suggest that, apart from providing energy to cellular processes, ATP functions as a "biological hydrotrope" at millimolar concentration. According to Neuberg (1916), a hydrotrope is a small, amphiphilic molecule which is capable of solving hydrophobic, normally insoluble compounds in water. A more detailed study by Mehringer et al. (2021) showed that ATP is indeed not a hydrotrope by Neuberg's terminology as it even decreases the solubility of small hydrophobic compounds in water. It rather exerts its solubilizing effects on proteins through a combination of (1) hydrophobic interactions between its adenine moiety and hydrophobic or aromatic amino acids exposed upon unfolding and (2) the strong solubilizing, kosmotropic effect of the triphosphate moiety (Figure 1). The kosmotropic Hofmeister effect refers to a strong hydration of the triphosphate moiety reinforcing the hydration shell of, e.g., a protein. This results in a reduced solubility but increased folding stability as hydrophobic interactions within the core of the protein are enhanced. However, in the case of ATP, the strongly kosmotropic triphosphate moiety is bound to a hydrophobic adenine moiety which can non-specifically bind to accessible hydrophobic sites, e.g., in an unfolded protein. While ATP is bound to its target via the adenine moiety, the strongly hydrated triphosphate moiety solubilizes the bound target (Mehringer et al. 2021), explaining the hydrotrope properties implicated by Patel et al. Further studies suggest that interactions between charged amino acids and the triphosphate moiety of ATP are also important (Kang et al. 2019; Tian and Qian 2021). In a similar way, ATP destabilizes RNA by adenine binding to unfolded states (Hautke et al. 2023) (Figure 2A). Using different NMR techniques, Nishizawa et al. further corroborated these points. They observed that ATP binds to both globular and intrinsically disordered proteins via transient, mainly hydrophobic interactions with nonpolar and/or aromatic amino acids. Further, they showed that ATP displaces water from the first hydration shell of a protein and replaces protein-water by protein-ATP hydrogen bonds (Nishizawa et al. 2021).

In total, intermolecular interactions between ATP and amino acids or nucleotides comprise charge-charge, π-cation or π-π-interactions and hydrogen bonds between the biomolecule and the adenine and triphosphate moieties of ATP. In very few cases, hydrogen bonds are also observed with the ribose moiety of ATP, but such interactions are limited to binding pockets in cases where ATP acts as a substrate or ligand (Babor et al. 2002; Lu et al. 2014). Prominent examples are shown in Figure 2B.

In this review, we categorize the manifold interactions of ATP as follows (Figure 1):

(1) ATP interacts as a specifically bound ligand or substrate to, e.g., enzymes.
(2) ATP interacts with the target molecule as a non-specific cosolute. Due to the different properties of the triphosphate and adenine moieties, there are two possible interaction models:
    (2.1) ATP acts as a kosmotropic Hofmeister salt by its triphosphate moiety.
    (2.2) ATP non-specifically interacts with a biomolecule by its triphosphate or the adenine moiety.

We discuss how these interactions modulate biomolecular processes in the following sections.

2 The effect of ATP on biomolecular folding stability

Patel and colleagues observed that ATP solubilized proteins such as FUS (fused in sarcoma; linked to amyotrophic lateral sclerosis (ALS)) and prevented or even reversed their aggregation. Similar findings were also reported for Amyloid β (Aβ) aggregates linked to Alzheimer's disease (AD) and aggregates formed by the prion-like domain of yeast protein Mot3 in the same study. When compared with hydrotropes used in industrial applications, it became apparent that ATP was a highly effective cosolute capable of solubilizing FUS at considerably lower concentration (Patel et al. 2017).

Gnutt et al. found that another protein linked to ALS, superoxide dismutase 1 (SOD1), was destabilized by ATP in a concentration-dependent manner. This observation is in line with a direct, non-specific binding (model 2.2) to hydrophobic residues, leading to a destabilization and increased solubility of the protein (Gnutt et al. 2019). Remarkably, SOD1 was stabilized up to an ATP concentration of 5 mM but destabilized at 10 mM and higher. This indicates that an ATP concentration higher than 10 mM could be detrimental for biomolecular processes in cells.

For proNGF, a precursor to the neurotrophic factor NGF (nerve growth factor), Paoletti et al. reported that ATP bound to an intrinsically disordered region via the adenine group, stabilized it and induced a conformational change, suggesting an allosteric modulation of the conformation of proNGF (model 1 and/or model 2.2). Further, they showed that proNGF binding to receptors $p75^{NTR}$ and TrkA was amplified and that binding to sortilin, another receptor, was diminished due to this conformational arrangement. Paoletti et al. reported that Mg^{2+} was competing with the receptors for the binding of ATP and that ternary Mg-ATP-receptor complexes were not observed. While NGF has a neurotrophic effect,

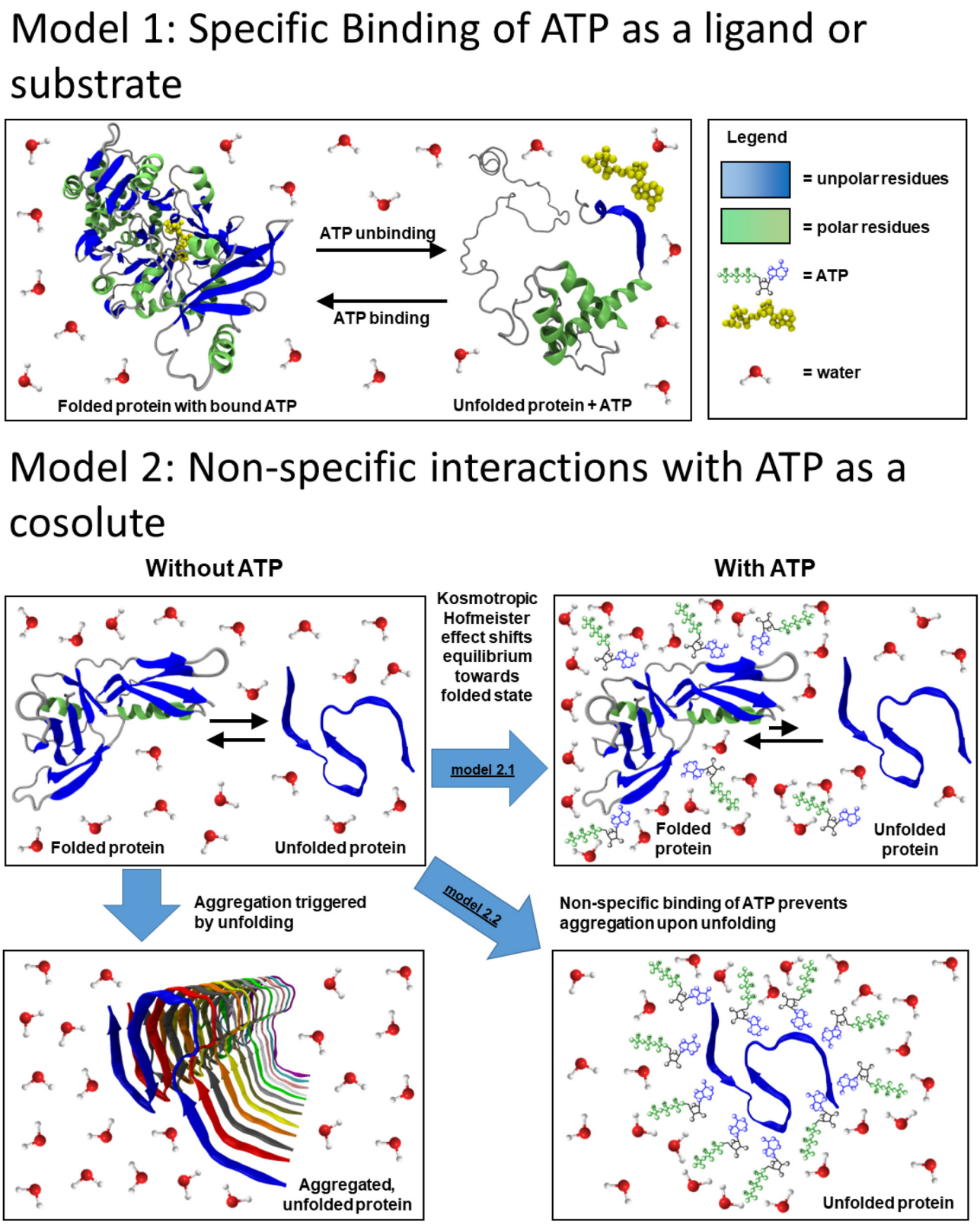

Figure 1: Different interaction modes of ATP as a cosolute. Model 1: ATP acts as a ligand or substrate for proteins and stabilizes the folded structure in the bound state. Crystal structures for illustration: D-alanine-poly (phosphoribitol) ligase subunit-1 bound to ATP (PDB: 3LGX) and unfolded PhoA (PDB: 2MLZ). Model 2: ATP enhances the folding stability of a protein due to the kosmotropic effect of the triphosphate residue (model 2.1) and prevents aggregation upon unfolding due to non-specific binding and solubilization of the unfolded state (model 2.2). Crystal structures for illustration: Amyloid ß precursor-like protein 2 (PDB: 7MQY) and amyloid ß/amyloid ß fibril (PDB: 2MXU).

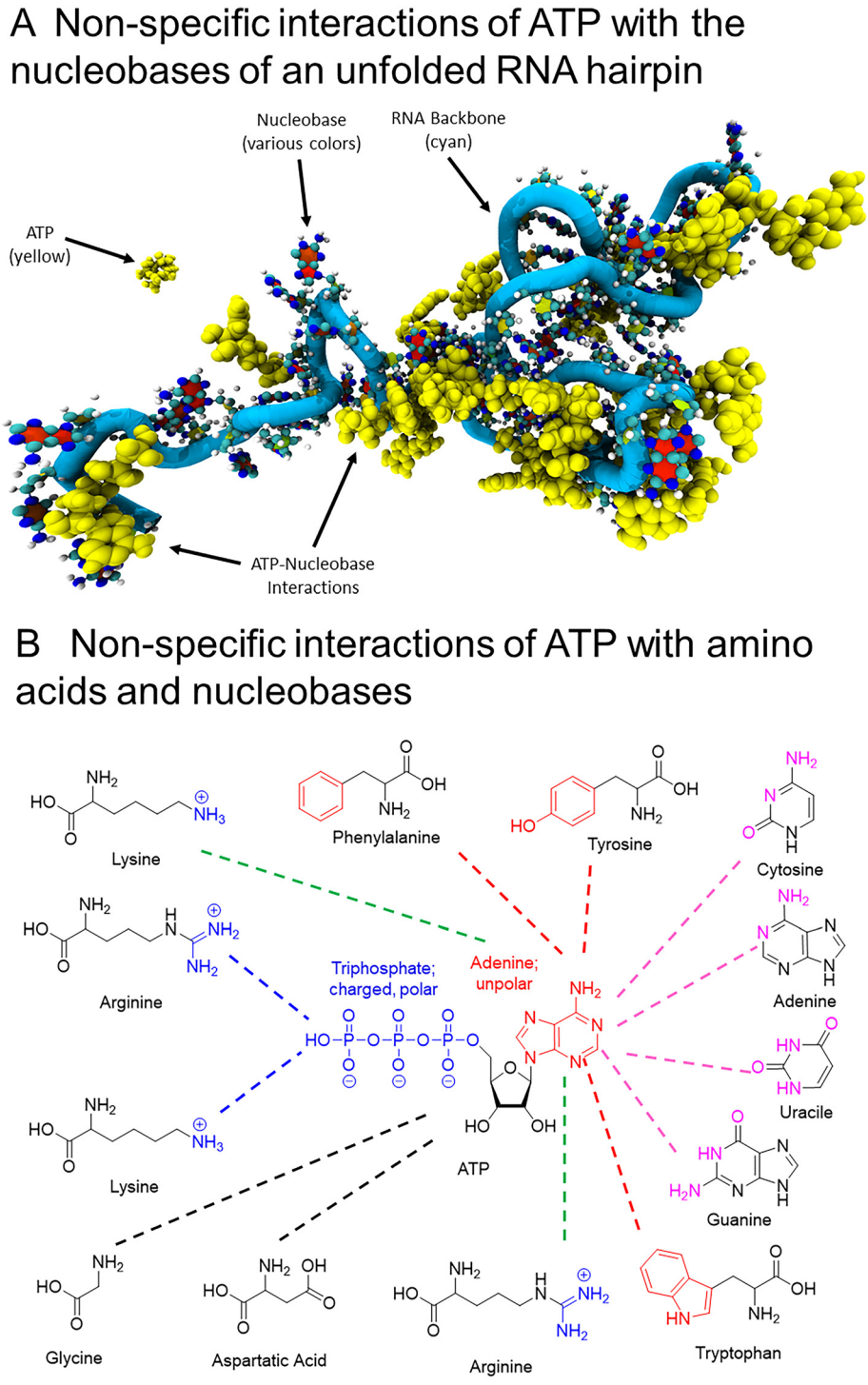

Figure 2: Non-specific interactions of ATP with different biomolecules. (A) Non-specific interactions of ATP (yellow) with an unfolded CAG-repeat RNA strand (cyan) via hydrogen bonding of the nucleobases with ATP (adapted from MD simulation results published in (Hautke et al. 2023). An unwound $(CAG)_{20}$ RNA hairpin was simulated in DPBS at 340 K and in presence of 5 mM ATP. The respective frame was taken from the simulation after 600 ns of simulation time. Comparisons between a folded and an unfolded hairpin at different temperatures and ATP concentrations revealed destabilization of the

proNGF is involved in neurodegeneration and neuronal apoptosis. proNGF maturates into NGF outside the cell at very low ATP concentration. Thus, binding of ATP might stabilize the precursor form inside the cell. Upon exocytosis into the extracellular medium, the intrinsically disordered region becomes destabilized in the absence of ATP. Thus, cleavage of this moiety during the maturation process is greatly facilitated.

Strong stabilizing effects of ATP were observed in cases where ATP acts as a ligand (model 1). For example, alanine scanning mutations of the ATP binding site of the enzyme APS kinase led to strong destabilizing effects (up to 19.2 kJ/mol) (Brylski et al. 2021). The absence of ATP as a substrate of the enzyme was assumed to lead to an unfolded and inactive state under ATP-depleted conditions such as starvation or DNA damage (Brylski et al. 2021). As such, the APS kinase activity could be directly responsive to ATP availability.

A proteome-wide study by thermal proteome profiling suggested that ATP could solubilize a considerable part of the insoluble proteome and specifically improve the solubility of positively charged intrinsically disordered proteins. The most significant stability shifts were observed for proteins which specifically bind ATP (model 1). At low ATP concentration (<500 μM), a shift was solely observed for those proteins which specifically bind RNA as a substrate. At intermediate concentration (1–2 mM), measurable effects were observed for larger protein complexes. At higher concentration (>2 mM), non-specific solubilizing effects by direct binding to protein monomers were observed (Sridharan et al. 2019).

For a CAG-repeat RNA hairpin related to Huntington's disease (HD), Hautke et al. (2023) reported that its folding stability was reduced by ATP in a concentration-dependent manner due to non-specific interactions between the adenine moiety of ATP and the nucleobases of the RNA in the unfolded state (Hautke et al. 2023). The CAG-repeat hairpin was significantly destabilized by ATP at millimolar concentration ($\Delta\Delta G_u^\circ$(DPBS + 10 mM ATP) = −8.2 kJ/mol). Such strong destabilization was otherwise only observed with small cosolutes like small PEG molecules or sucrose (Hautke and Ebbinghaus 2021) at 50- to 100-fold higher concentration.

A combination of crowding agents and ATP at physiological concentration allowed to reproduce the measured in-cell stability (ΔG_u°(HeLa cell) = 6 − 7 kJ/mol). Surprisingly, ATP concentrations above the physiological range (15, 20 mM) led to rapid, amorphous aggregation of the RNA. This provides further evidence that there could be an upper limit to ATP concentration in cells above which a further increase might be detrimental to biomolecular processes.

In the same study, another hairpin RNA derived from the 4U RNA thermometer was investigated. Remarkably, it turned out that ATP stabilized this hairpin at low concentration but destabilized it at higher concentration in dilute buffer solution ($\Delta G_f^{\circ,37\,°C}$(DPBS) = −0.3 kJ/mol; $\Delta G_f^{\circ,37\,°C}$(DPBS + 5 mM ATP) = −1.3 kJ/mol; $\Delta G_f^{\circ,37\,°C}$(DPBS + 10 mM ATP) = +1.2 kJ/mol). The initial stabilizing effect could be attributed to the kosmotropic Hofmeister effect of ATP on the folded state while the destabilizing effect at higher ATP concentration could be explained by non-specific binding of ATP to the nucleobases via the adenine moiety.

The studies reviewed above report a considerable and, at times, even striking impact of ATP on biomolecular folding stability. Yet, many experiments were only carried out *in vitro*, with an unknown ability to reproduce physiologically relevant behavior in the cell, where manifold interactions with folded or unfolded states change the folding equilibrium. Macromolecular crowders stabilize proteins entropically via excluded volume or water-mediated effects (Minton 1981; Rivas and Minton 2016; Senske et al. 2016). Quinary and non-specific interactions destabilize proteins by interactions with the protein surface (Gnutt et al. 2019; Monteith et al. 2015). Salts, ions or osmolytes can directly interact with protein surfaces (Senske et al. 2014; Street et al. 2006) but also lead to osmotic effects (Gnutt et al. 2017). Additionally, biomolecular processing such as posttranslational modifications or chaperone interactions can shift the folding equilibrium (Wood et al. 2018). These effects must be additionally considered when extrapolating the *in vitro* ATP studies onto the cellular level, noting that an altered folding equilibrium in the cell may lead to different ATP interactions. One way to test the effects of ATP directly inside the cell are ATP-depletion experiments (Brylski et al. 2021; Hautke et al. 2023). Such experiments qualitatively resemble corresponding test tube experiments, but need to be interpreted carefully since secondary effects may complicate the analysis. For instance, the refolding or holdase activity of chaperones is mostly dependent on ATP. Thus, decreased cellular ATP concentrations will also mitigate chaperone-driven homeostasis mechanisms (Lindberg et al. 2015;

RNA hairpin by increased non-specific base-base interactions between RNA and ATP in the unfolded state). (B) Non-specific intermolecular interactions between ATP and different amino acids and nucleobases. With cationic amino acids, ATP can interact either electrostatically with the triphosphate moiety or via π-cation interactions with the adenine moiety. With aromatic amino acids, π-π-interactions are possible. Interactions with nucleic acids occur mostly via hydrogen bonding. In special cases (mostly within binding pockets), hydrogen bond interactions are also possible between an amino acid and the ribose moiety.

Tittelmeier et al. 2020). Decoupling such secondary effects from cosolute mechanisms remains difficult on the cellular level.

3 The role of ATP in phase separation

Since ATP governs the stability and solubility of RNAs and proteins, it is expected that it also influences self-assembly processes like aggregation and phase separation. In fact, ATP triggers and/or represses liquid-liquid phase separation (LLPS) in a concentration-dependent manner due to electrostatic interactions.

One example for such behavior was FUS for which ATP promoted LLPS at low concentration but dissolved droplets at higher concentration. ATP non-specifically bound to cationic arginine (R) and lysine (K) and aromatic residues through π-π- and π-cation interactions between the amino acids and the adenine residue as well as electrostatic interactions between the amino acids and the triphosphate moiety (Figure 2). Thereby, ATP mitigated charge-charge repulsions at low concentration, promoting LLPS. Above a certain ATP concentration, the protein monomers were highly solubilized, preventing LLPS and leading to the solvation of phase-separated droplets (Figure 3) (Kang et al. 2018).

A similar behavior was observed for the RNA-driven LLPS of the cold-shock protein CIRBP which contains disordered R–K repeat regions similar to those of FUS. RNA promoted LLPS at low concentration and repressed it at higher concentration with a similar mechanism. ATP competed for the RNA binding sites and repressed LLPS of the RNA-protein mixture in a concentration-dependent manner (Zhou et al. 2021).

A third example for ATP mediating LLPS by tweaking electrostatic interactions was given by an IgG antibody (Tian and Qian 2021): Rapid LLPS was only observed upon addition of ATP under acidic conditions under which the antibody was highly positively charged. In this case, the authors also attributed the onset of LLPS to reduced repulsive interactions due to the negatively charged triphosphate moiety of ATP. LLPS was suppressed at higher pH and did not occur after adding ADP, AMP or adenine to the solution. This finding further supports the importance of the strong negative charge of the triphosphate moiety for the electrostatic interactions governing LLPS. Further, adding Na^+ or Mg^{2+} to the solution quickly dissolved phase-separated droplets by screening the negative charge of ATP.

In a very recent study, Dec et al. (2023) showed that the phase behavior of a mixture of ATP and the insulin-derived oligopeptide $ACC_{1–13}K_n$ (n = 8, 16, 24, 32, 40) was tuned by electrostatic interactions. The alanine-cysteine ($ACC_{1–13}$)

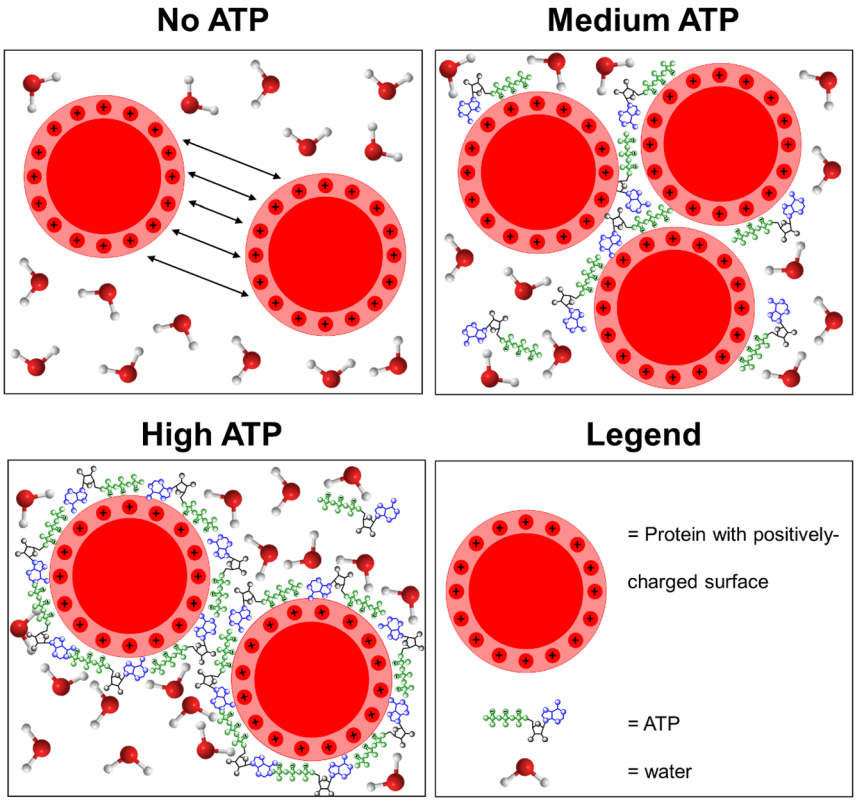

Figure 3: Effect of ATP in LLPS of positively charged proteins. ATP promotes or represses LLPS in a concentration-dependent manner (Kang et al. 2019; Tian and Qian 2021; Zhou et al. 2021). Without ATP the protein does not undergo phase separation due to charge-charge repulsions. At intermediate ATP concentration these repulsions are mitigated by the negative charge of the triphosphate moiety. At high concentration ATP prevents LLPS due to the strong solvation of the protein monomers.

moiety was found hydrophobic and aggregated spontaneously. K_n, on the other hand, was positively charged and formed droplets in the presence of ATP without any aggregation. When put together in a chimeric oligopeptide, $ACC_{1–13}K_n$ stoichiometrically co-aggregated with ATP and formed fibrils in which ATP became selectively incorporated. The phase behavior of such chimeric peptide was governed by electrostatic interactions. For short K_n tracts (n = 8, 16), fibrillation occured right away. However, for n = 24, 32 and 40, $ACC_{1–13}K_n$ formed liquid droplets prior to fibrillation. Pressure perturbation experiments showed that droplets and fibrils formed due to charge-charge interactions between ATP and the K_n tract and were stabilized by hydrogen bonds between $ACC_{1–13}$ moieties. Thus, ATP-driven charge-charge interactions leading to droplet formation can at least temporarily outcompete hydrophobicity-driven fibril-lation, providing another example for the solubilizing properties of ATP.

In another study, Mahapatra et al. (2023) reported that the aggregation of the positively charged, prion-like N-terminal and middle domain (NM) of yeast protein Sup35 was promoted by high ATP concentration. However, this effect was not observed at lower ATP concentration and/or in the absence of Mg^{2+}. The authors attributed these results to charge-charge interactions between ATP-Mg and positively charged residues in NM. Additionally, ATP disaggregated previously formed NM fibrils by fragmenting them and promoted the formation of NM amyloids uncapable of seeding further aggregation.

Similarly, Kota and colleagues reported that ATP drove phase separation of the basic intrinsically disordered proteins (bIDPs) poly-lysine (pK) and protamine (PM; rich in arginine) over a wide range of ATP concentrations including the physiological range (Kota et al. 2023). At higher concentration, first amorphous aggregation (>500 mM) and then fibrilation (>750 mM) were triggered. Further, the authors found that ATP was highly enriched inside bIDP droplets, leading to a protein network with ATP forming protein bridges. Simulations elucidated that ATP mediates the interaction of the protein molecules by hydrogen bonds, π-cation and π-π interactions. This study is another example that ATP can modify LLPS in different ways, depending on its biomolecular interaction mechanism.

In the previously described studies, the effect of ATP on LLPS was attributed to electrostatic interactions. In other examples the mechanism is less clear. Brangwynne and coworkers observed that ATP depletion increased the viscosity of nucleoli in *Xenopus laevis* oocytes, changing from liquid to almost solid behavior (Brangwynne et al. 2011). Further, the authors showed that certain proteins such as nucleophosmin and fibrillarin were selectively retained within physiological aggregates inside nucleoli extracted from *X. laevis* oocytes (Hayes et al. 2018). These aggregates became partially or even fully solubilized in the presence of the soluble protein fraction of the nucleoli and ATP in an ATP concentration-dependent manner. Also, hydrolysis of ATP was required to dissolve the aggregates. On the other hand, aggregates behaved insoluble after depletion of soluble proteins and small solutes such as ATP from the nucleoli. Thus, it was assumed that an energy-consuming first step involving, e.g., a soluble protein chaperone was necessary to afterwards enable solubilization of the aggregates by the cosolute effect of ATP.

In the previously described study by Hautke et al. (2023), the authors found that the CAG-repeat RNAs recruited to nuclear speckles were mobile under physiological conditions, but fully immobilized upon depletion of ATP. This effect could be attributed to either a lack of energy supply for RNA chaperones and/or entanglements with either other RNA molecules or hairpin-binding proteins (Hautke et al. 2023; Jain and Vale 2017).

Jain and colleagues found that ATP is necessary for the formation and dynamic behavior of stress granules formed by G3BP (Jain et al. 2016). Depletion of ATP from the cell completely prevented formation of stress granules and inhibited their movement as well as fusion events. Liquid-like behavior was also significantly reduced.

The eye lens, one of the most crowded environments in the human body (total protein concentration = 400 g/L; even higher in other organisms), is another intriguing example. The optical properties, especially the high refractive index, can be attributed to a family of proteins named crystallins which make up the biggest share of proteins within eye lens cells. Interestingly, crystallins were found to be neither recycled nor reproduced and are sustained for the organism's entire lifetime. The aggregation or phase transitions of these proteins would lead to a cataract. Although the metabolic activity of the eye lens is generally low, an ATP concentration of 3–7 mM is constantly maintained, possibly to prevent crystallins from aggregation in this highly crowded environment via the kosmotropic effect of the triphosphate moiety (Song 2021).

In summary, the ATP effects can be attributed to electrostatic and π-cation interactions for highly charged proteins leading to a concentration-dependent enhancement or suppression of LLPS. In other cases, where electrostatic interactions do not dominate, a molecular interpretation of the data is mostly lacking. To interpret these studies, experiments and simulations using reconstituted two-component model systems are needed to quantitatively evaluate the cosolute mechanism (Senske et al. 2014, 2016).

4 Implications for neurodegenerative diseases

Since biomolecular folding stability and phase separation play a role in neurogenerative diseases, it is intriguing to consider a possible role of ATP in disease mechanisms. For the case of AD, mitochondrial dysfunction manifested as a decrease in neuronal ATP levels and an overproduction of reactive oxygen species (ROS) (Misrani et al. 2021).

Both amyloid precursor protein (APP) and its proteolytic product Aβ were observed to bind to the α subunit of ATP synthase and colocalize with it at the cellular surface of neurons, promoting its migration to the cell membrane. However, binding of APP or Aß by the α subunit at the cellular membrane inhibited the ATPase activity of the F_0F_1 ATP synthase complex, leading to an inhibition of the hydrolysis of extracellular ATP. Also, extracellular ATP production was reduced. A consequence of this was an impairment of synaptic plasticity as shown by an inhibition of both short-term and long-term potentials (Schmidt et al. 2008).

Patel et al. found that ATP was capable of dissolving Aβ fibrils *in vitro* (Patel et al. 2017). In line with these findings, Ebanks et al. reported that the drug candidate J147 was capable of rescuing cognitive defects typical for AD in mouse models by modulating the enzymatic activity of the α-subunit of ATP synthase (Ebanks et al. 2020). These findings depict two possible disease-related mechanisms for amyloid ß (Aß) in which ATP could play a role: (1) Aß affects the production of ATP by inhibiting ATP synthesis and misplacing the α subunit to the cell membrane where it cannot be used for intracellular ATP production. (2) The resulting cellular ATP depletion enhances formation of Aß fibrils in a self-amplifying mechanism. As such, the maintenance of cellular ATP levels, e.g., by drugs that increase ATP production, appears to be a possible strategy for therapeutic intervention.

In the muscles of patients suffering from Parkinson's disease (PD), ATP production was also found decreased (Mischley et al. 2023). Inhibition of complex 1 of the OXPHOS chain as observed in PD patients led to an impairment of mitochondrial respiration and, thus, decreased ATP production and dramatically increased ROS production. Another effect of complex 1 inhibition was the specific degeneration of dopaminergic neurons, which is a central feature of PD. However, it was found that an inhibition of OXPHOS complex 1 of at least 50 % was needed to impair ATP production, but the extent of inhibition observed in PD patients was only 25–30 %. (Perier and Vila 2012). Yet, Nakano et al. (2017) reported several drug candidates such as esculetin which acted as agonists towards estrogen receptor-related receptors (ERRs) and elevated cellular ATP levels by either upregulating ATP production or reducing its consumption. Treatment with these compounds rescued disease features of PD (among them ATP depletion) and prevented apoptosis in PD cell models. Further, treatment of cells with esculetin fully prevented accumulation of α-synuclein and rescued behavioral disease features in mouse models of PD. These findings seem relatable to the ones described above for AD. In both cases, ATP depletion due to a hampered protein within the ATP production chain is reported and central disease features including aggregation of α-synuclein are remedied by drugs which upkeep cellular ATP levels. Thus, this strategy also seems to be beneficial for treating PD.

Another disease condition in which ATP could play a role is type 2 diabetes mellitus (T2DM). Human islet amyloid polypeptide (hIAPP) folding into β-sheets and the resulting formation of fibrils is associated with disease onset. Simulations showed that the β-sheet content in hIAPP is reduced, and that the formation of random coils was increased in the presence of ATP in a concentration-dependent manner. ATP prevented the formation of β-sheets by shielding the monomers from one another (Roy and Paul 2021).

In HD, striatal neurons are affected by mutant huntingtin (mHTT) protein (Damiano et al. 2010; Jodeiri Farshbaf and Ghaedi 2017). In general, neurons are cells with high metabolism and, thus, high demands in ATP. Yet, it was found that striatal neurons were outstandingly susceptible to decreased cellular ATP levels (Damiano et al. 2010). Physiological HTT interacts with the mitochondrial membrane, governs fusion and fission events which are essential for mitochondrial function and regulates transport of mitochondria along the axon in both directions. Expression of mHTT affected mitochondria and led to their inhomogeneous distribution throughout the cell, decreased cellular ATP levels and, finally, apoptosis (Damiano et al. 2010; Jodeiri Farshbaf and Ghaedi 2017). Monkey models of HD showed signs of mitochondrial dysfunction such as elevated lactate levels in brain and muscle cells. Also, inhibition of oxidative phosphorylation lead to selective damage of neurons in the striatum as observed in HD (Lieberman et al. 2019).

Although no clear proof of ATP preventing aggregation of mHTT and/or dissolving previously formed aggregates has been reported in literature yet, there were several pieces of evidence pointing in this direction: Yeast cells with deficient ATP homeostasis mutants were found very sensitive to aggregation-prone mHTT and perished upon its expression (Takaine et al. 2022). Overexpression of ATP synthase α considerably reduced production of mHTT in SH-SY5Y cells and improved cell viability (Wang et al. 2009). These findings make a similar aggregation-preventing and disease-remedying effect of ATP on mHTT as is reported for Aβ, α-synuclein and hIAPP appear reasonable.

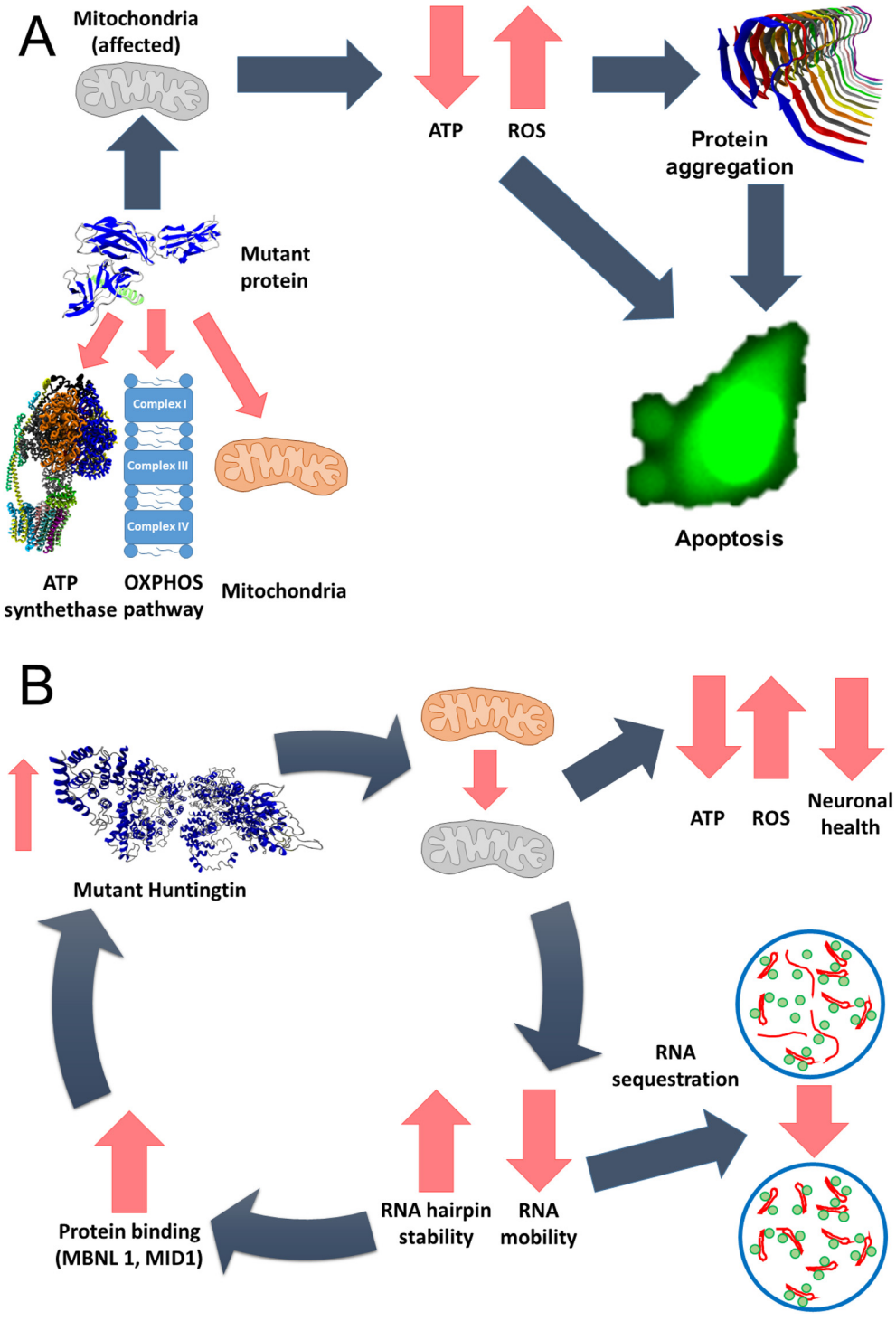

Figure 4: Involvement of ATP in neurodegenerative diseases. (A) Possible disease-relevant mechanisms involving ATP and mitochondria for the cases of AD, PD and type 2 diabetes mellitus. Mutant proteins interact with the OXPHOS (oxidative phosphoryllation) pathway, ATP synthetases or mitochondria in a detrimental manner. As a consequence, mitochondria are affected, resulting in decreased cellular ATP levels and increased ROS production, leading to aberrant aggregation or fibrillation of proteins and apoptosis of susceptible cells. Crystal structures for illustration: ATP synthetase (PDB: 5FIL), complex of contactin 4 with amyloid ß precursor-like protein 2 (PDB: 7MQY) and amyloid ß fibril (PDB: 2MXU). (B) Possible disease mechanism involving ATP and mitochondria in the case of HD: mHTT interferes with mitochondria, impairing them and leading to increased ROS and decreased ATP production. Due to declining cellular ATP levels, the folding stability of RNA hairpins is increased, and their mobility is decreased. The increased folding stability enhances the interaction with RNA-binding proteins such as MBNL1 and MID1, resulting in RNA sequestration and upregulated mHTT expression, a self-amplifying mechanism. Crystal structure for illustration: full-length mHTT (PDB: 6YEJ).

In general, correlation of cell death and reduced ATP levels is a common feature in many (neurodegenerative) diseases (Nakano et al. 2017). Also, impairment of mitochondria leading to abnormal mitochondrial dynamics (fission and fusion), excessive ROS production, loss of Ca^{2+} homeostasis and decreased ATP production is a reoccurring feature in HD, AD and PD (Damiano et al. 2010; Jodeiri Farshbaf and Ghaedi 2017; Misrani et al. 2021; Perier and Vila 2012). Remarkably, several studies report that the maintenance of cellular ATP levels can rescue the respective symptoms of these diseases (Ebanks et al. 2020; Nakano et al. 2017; Wang et al. 2009). Many drug candidates which are tried as potential remedies for AD and PD show, among others, activity towards the upkeep of cellular ATP levels and the reduction of ROS production (Fernández-Moriano et al. 2015). For PD cell and rodent models, ATP production-maintaining or even -increasing effects were reported for substances from many different classes such as the hormone melatonin, the polyphenol resveratrol, the flavonoid baicalein or the carotenoid lycopene. Similar results were reported for AD with the mitochondrial division inhibitor mdivi-1 and the alkaloid caffeine. Yet, a detailed mechanism was not discussed for any of these cases.

In summary, a common underlying pathogenic mechanism involving the function of ATP as a cosolute could be as follows. At first, disease-specific stimuli, e.g., interaction with proteins such as Aβ or mHTT impair the function of mitochondria through various pathways. This leads to detrimental effects such as decreased motility and fusion/fission events, increased ROS production and a decrease of cellular ATP levels. In the following, certain proteins form aggregates, amyloids and/or fibrils or phase-separate in an aberrant manner, the folding stability of proteins and RNAs is altered, and the viscosity of the cellular medium is drastically changed, tipping the cellular machinery out of homeostasis. Under physiological conditions, these detrimental processes are prevented by physiological ATP concentration. Finally, depending on the expression pattern of the disease-relevant proteins, the specific disease mechanism, and the susceptibility of host cells to detrimental factors such as ATP depletion or increased ROS production, specific cell types perish and cause symptoms.

In cases such as HD where both mHTT and its corresponding mRNA are presumably involved in disease pathology, self-amplifying mechanisms could evolve: Reduced cellular ATP levels lead to increased stability of the mRNA's CAG-repeat hairpin (Hautke et al. 2023) which, presumably, increases binding to hairpin-binding translation and transcription factors such as MBNL1 (Wojciechowska and Krzyzosiak 2011) or MID1 (Krauss et al. 2013). In the following, translation of mHTT is increased. This affects mitochondrial function, leading to a further decrease in cellular ATP levels (Damiano et al. 2010; Jodeiri Farshbaf and Ghaedi 2017). These mechanisms are further illustrated in Figure 4.

5 Conclusions

In summary, we conclude that ATP is an important cosolute governing biomolecular folding stability, phase separation and aggregation. It modulates biomolecular folding stability by specific (model 1) as well as non-specific binding (model 2.2) and as a kosmotrope (model 2.1). Depending on the concentration and the biomolecular properties, ATP exerts an either promoting or repressing effect on LLPS and aggregation. As such, ATP plays a role in several neurodegenerative diseases preventing the aggregation of proteins or dissolving previously formed aggregates. Restoring cellular ATP levels rescued disease features in AD, PD and HD models, motivating further research in this direction.

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