News & Views

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Behind closed gates – chaperones and charged residues determine protein fate

Margreet B Koopman[1,2] & Stefan GD Rüdiger[1,2]

Charged residues flanking aggregation-prone regions play a role in protein folding and prevention of aggregation. In this issue of *The EMBO Journal*, Houben *et al* exploit the role of such charged gatekeepers in aggregation suppression and find that negative charges are more effective than positive ones. Strikingly, the prominent Hsp70 chaperone has a strong preference for the less effective, basic gate keepers. This implies co-adaptation of chaperone specificity and composition of protein sequences in evolution.

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See also: **B Houben *et al*** (June 2020)

Folding into the native state is for many proteins a prerequisite for proper function. A key building principle of the native state is that hydrophobic residues fold inside the protein and constitute the hydrophobic core. Folding of a protein resembles Stevenson's novella featuring the kind Dr. Jekyll who transforms into the mischievous Mr. Hyde by drinking a magic serum. Proteins resemble Dr. Jekyll when they correctly assemble their hydrophobic stretches inside its core. Failure to do so, however, transforms the protein into the evil Mr. Hyde, exposing aggregation-prone regions (APRs), which leads to misfolding and potentially toxic aggregates (Rousseau *et al*, 2006). Small single-domain proteins fold fast and effectively into Dr. Jekyll. However, in particular large multi-domain proteins tend to deviate from the productive folding path and may turn into Mr. Hyde instead. A network of molecular chaperones ensures proper folding and prevents potentially evil transformation of protein structures (Dahiya & Buchner, 2019).

Key factors in this network are the conserved, ATP-driven Hsp70 and Hsp90 chaperone machines (Morán Luengo *et al*, 2019). These two most abundant chaperone systems form a cascade; Hsp70 acts early on the folding path, further unfolds its protein substrate and subsequently delivers it for further maturation to the Hsp90 system. The chaperones do not actively fold the protein, and they empower it to fold on their own into the native conformational state as determined by their primary sequence. Hsp70 specifically recognises a sequence motif that is trademark for the composition of the hydrophobic protein core. Such sites typically have three or more large hydrophobic or aromatic residues in a short stretch of four to five residues that are particularly enriched in leucine (Rüdiger *et al*, 1997). The flanking regions of these hydrophobic segments are enriched in positively charged residues, which correlate with the negative potential of the surface of the Hsp70 substrate binding domain. The importance of basic residues is remarkable as they are an intrinsic feature of the hydrophobic core, and their importance for chaperone function has been poorly understood so far.

Intriguingly, positively charged residues in the flanking regions of Hsp70 binding sites mirror the finding that charged residues often cap the N- and C-terminal ends of APRs. These charges act as so-called gatekeepers (GKs) counteracting the aggregation propensity of the APRs (Otzen & Oliveberg, 1999; Rousseau *et al*, 2006; Beerten *et al*, 2012). Does it now matter whether a GK is positively or negatively charged? And why would Hsp70 chaperones have a preference for positively charged GKs? In this issue of The EMBO Journal, Houben *et al* provide experimental answers to these questions, merging the GK concept and the chaperone paradigm (Houben *et al*, 2020). The authors provide for a functional explanation for the specific taste of Hsp70 for basic residues and consider their findings in the context of co-evolution of chaperone specificity and protein composition.

A key observation by Houben *et al* is that the nature of the GKs determines efficiency in prevention of aggregation. They assess the impact of specific sequence features on aggregation. Such charged flanking residues kinetically disfavour amyloid-like fibril formation because of Coulomb repulsion by their sidechains. Remarkably, negatively charged residues are more efficient as GKs than positively charged residues. In fact, acidic sidechains are less able to intrude deeply into the hydrophobic core than basic residues. This drive for the surface may make acidic residues more effective in preventing aggregation than basic residues, which are less capable of maintaining protein solubility by themselves.

Houben *et al* compare GK properties to the determinants of Hsp70 specificity. Strikingly, Hsp70 substrates are enriched in vulnerable APRs flanked by basic residues—*i.e.* APRs less effective in gatekeeping by themselves, suggesting possible co-evolution of chaperone specificity and the foldable proteome (Fig 1). Negative selection on aggregation-prone proteins may correlate with adaptation of molecular chaperones to amino acid sequences coming through in evolution (Rousseau *et al*, 2006). Turning this around, proteins may have been adapted to the conserved chaperone pool. Poorly adapted proteins may thus never reach the native state, as they would suffer more protein misfolding resulting in enhanced functional impairment at cellular level.

As a functional cell relies on functional proteins, the purpose of the folding pathway

1 Cellular Protein Chemistry, Bijvoet Centre for Biomolecular Research, Utrecht University, Utrecht, The Netherlands
2 Science for Life, Utrecht University, Utrecht, The Netherlands
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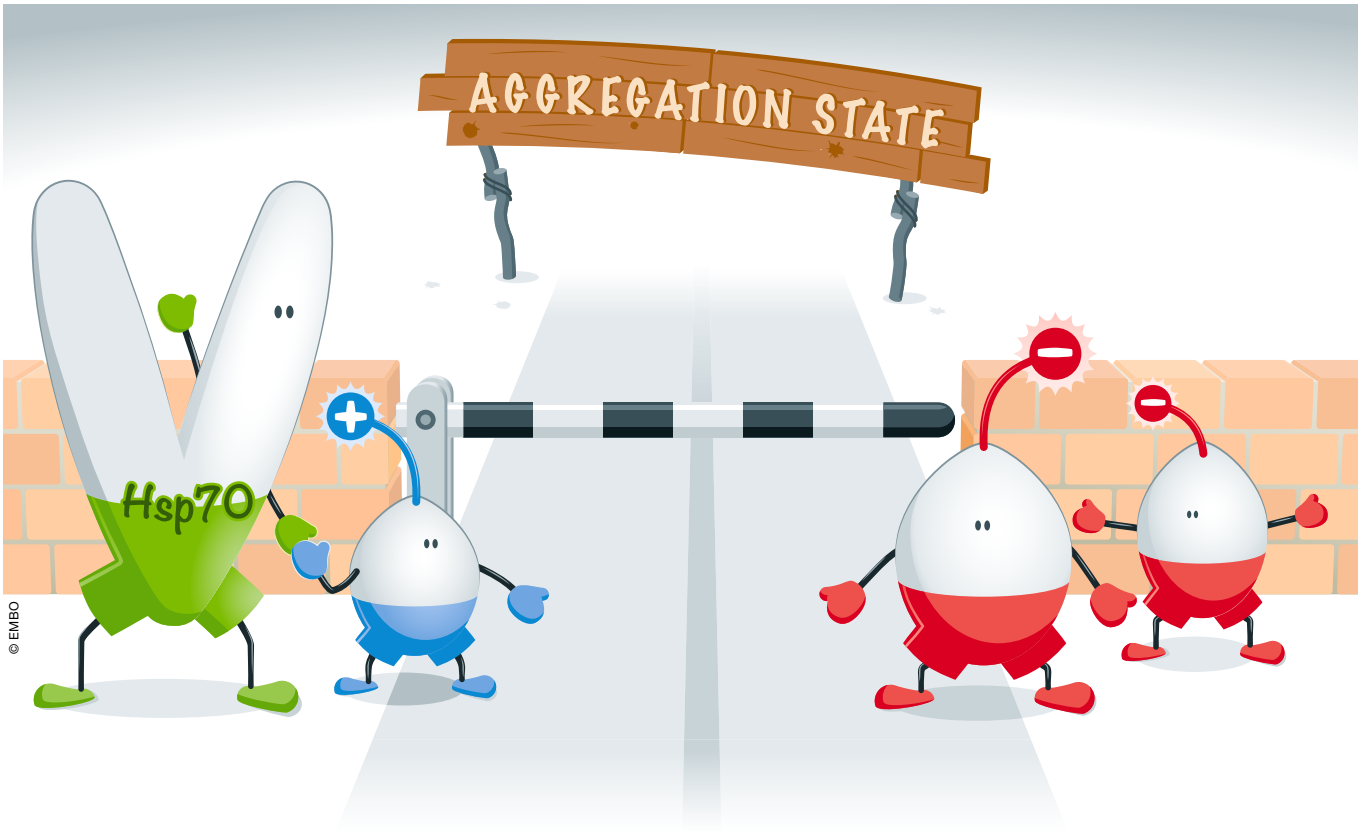

Figure 1. Charged residues and molecular chaperones guard the gate of the road to aggregation.

Schematic representation of the gatekeeper concept taming aggregation-prone regions. Negatively charged (red) and positively charged residues (blue) cap both ends of hydrophobic stretches, which limit misfolding and aggregation and support the productive folding pathway towards the native state. Negatively charged residues are sufficiently powerful on their own, and positively charged residues are supported by Hsp70 chaperone machine (green).

is not merely preventing aggregation but effectively ensuring that all proteins adopt their native fold. In that light, aggregation prevention is a side-effect of chaperone-guided protein folding. Aggregation relies on aberrant intermolecular pairing of hydrophobic stretches, while misfolding occurs because of fatal intramolecular interactions of such stretches. Thus, charged GKs may improve controlled protein folding by cutting off such dead-end pathways. In fact, proteins without charged GKs would even fold faster, but with increased risk for aggregation as a trade-off (Kurnik *et al*, 2012). Blocking unproductive pathways by GKs will be most important in the early folding stages before the hydrophobic core has been formed. Importantly, this is also the phase when Hsp70 chaperones are most active (Morán Luengo *et al*, 2018).

Affinity for positively charged GKs is not restricted to Hsp70, and they are also crucial for binding by DnaJ, SecB and trigger factor (Knoblauch *et al*, 1999; Patzelt *et al*, 2001; Rüdiger *et al*, 2001). These chaperones have more open substrate binding sites than Hsp70s, making positioning of specific residues in sequence motifs less stringent. This remarkable joint taste for basic GKs makes substrate specificity of all cytosolic chaperones compatible with each other. This is exemplified for the aggregation-prone proteins Tau and α-synuclein in which binding sites of several chaperones overlap (Karagöz *et al*, 2014; Burmann *et al*, 2020). Tau and α-synuclein are both intrinsically disordered proteins (IDPs), and both form neurotoxic fibrils. Although both lack defined tertiary structure, they have Hsp70 binding sites with positively charged residues as GKs in their flanking regions. This suggests that the GK concept also applies to IDPs, which cannot hide their hydrophobic stretches inside a core.

Together, Houben *et al* not only provide an explanation for the presence of charged residues in flanking regions of APRs, they also elucidate the preference of molecular chaperones for basic over acidic residues (Houben *et al*, 2020). These findings shed new light on how molecular chaperones are specifically adapted to such protein segments that are most vulnerable for dead-end misfolding reactions—and these include positively charged residues.

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