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Many features of the structure and mechanism of the prokaryotic group I chaperonin GroEL/GroES have been elucidated. Archaea and eukarya use the related Group II chaperonins, with a roughly homologous double barrel structure, but opening and closing without an additional small subunit. Previous research has shown that group II chaperonins preferentially associate with β-sheet proteins. Of particular interest is how the group II chaperonins distinguish their partially folded substrates from the native state.

We have employed the homo-oligomeric archaeal group II chaperonin of *Methanococcus maripaludis* (Mm-Cpn) to study the recognition of the partially folded β-sheet substrate protein Human lens γD-Crystallin (HγD-Crys). All members of the βγ-crystallin family exhibit a double Greek key fold, in duplicated domains. Candidates for recognition motifs including the domain interface, paired aromatics, oxidatively damaged residues, and β-hairpin strands of the buried core. Mutations of residues participating in these motifs have been shown to destabilize HγD-Crys. To assay for chaperonin recognition of mutant chains, we monitored suppression of off-pathway aggregation upon dilution out of denaturant, in the presence or absence of chaperonin. The amount of HγD-Crys refolded back to the native state by Mm-Cpn was also quantified.

The aromatic mutants Y92A, Y97A, and the double mutant Y92A/Y97A are destabilized compared to WT HγD-Crys (thermal melting of 77.0 ± 0.2, 79.0 ± 0.2, and 75.6 ± 0.2, respectively, compared to 83.8 ± 1.3 °C). If these normally buried aromatic residues are a signal for chaperonin recognition, the absence of these aromatic residues might reduce recognition by Mm-Cpn. Aggregation of these HγD-Crys mutants in the presence and absence of Mm-Cpn was measured. While Mm-Cpn suppressed the unpaired aromatic HγD-Crys mutants to the same level as WT HγD-Crys, the aggregation of the mutants in the presence of Mm-Cpn was much slower. This indicated that Mm-Cpn recognized the mutant species better than it recognized WT HγD-Crys aggregation intermediates. Furthermore, Mm-Cpn refolded 40-45% of the unpaired aromatic mutant HγD-Crys compared to 20% of WT HγD-Crys. Not only did Mm-Cpn recognize these mutants better, but it refolded them more efficiently than it refolded WT. From these sets of mutants we can conclude that Mm-Cpn is not recognizing the aromatics themselves, but rather a feature of the Greek key β-sheet that is exposed in folding intermediates in the absence of these aromatic residues.