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The importance of unstructured termini in the aggregation cascade of beta-2-microglobulin: insights from molecular simulations of D76N mutant

Rui João Loureiro [a], *D.V. Viçosa*[b], *M. Machuqueiro*[b], *Eugene I. Shakhnovich*[c], Patrícia F.N. Faísca[a,d].

[a] *BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências da Universidade de Lisboa*

[b] *Centro de Química e Bioquímica, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade de Lisboa*

[c] *Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts*

[d] *Departamento de Física, Faculdade de Ciências da Universidade de Lisboa*

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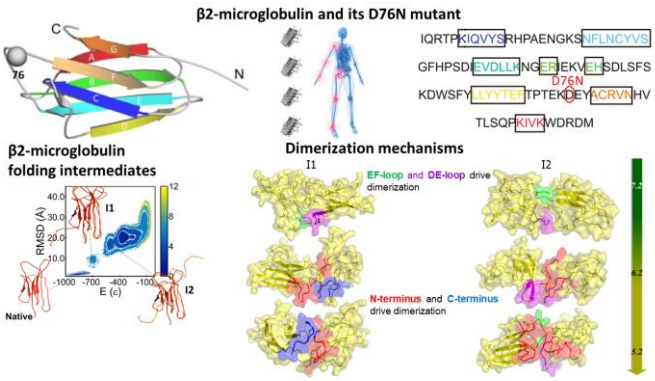
Graphical Abstract



Abstract.

The identification of folding and aggregation intermediate states is important, both from a fundamental standpoint and for the design of new therapies for conformational disorders. Here, we use the single point mutant (D76N) of β2m, the causing agent of a hereditary systemic amyloidosis affecting visceral organs, as a model system to study the aggregation mechanism of β2m using molecular simulations. We present our predictions on the early molecular events triggering the amyloid cascade for the D76N mutant. Folding simulations highlight the existence of an aggregation-prone intermediate called I1 which presents an unstructured C-terminus and of an aggregation-prone intermediate featuring two unstructured termini called I2. Additionally, Monte Carlo docking simulations suggest that both intermediates have high aggregation-propensity. These simulations support an essential role of the termini and of the DE and EF-loops in the

	dimerization of both intermediates. The relevance of the C-terminus is higher at the acidic pH 5.2 while the N-terminus become more important at pH 6.2. At physiological pH, the DE and EF-loops are the most important regions for dimerization. These predictions rationalize experimental results that support the involvement of Lys-19, Phe-56, Trp-60 and Phe-62 in amyloidogenesis in the wild-type and other model systems of β2m.

Introduction *(optional)*

The identification of a folding intermediate state of the protein beta-2-microglobulin (β2-m) that is also able to trigger the aggregation cascade [1] [2] showed that folding and aggregation may be competing processes, and that the identification of intermediate states for folding and aggregation is essential both to understand the mechanisms of protein aggregation and to design new therapeutic strategies targeted at conformational disorders. A well-known example is dialysis related amyloidosis (DRA) [3], a fatal condition that affects ~700000 individuals worldwide with chronic renal failure undergoing long-term hemodialysis, characterized by the deposition of β2-m amyloid fibrils in the osteoarticular tissues.

Recently, a single point mutant of β2-m (D76N) was identified as the causing agent of a hereditary systemic amyloidosis affecting visceral organs [4]. This mutant is considerably more amyloidogenic *in vitro* at physiological conditions than the wild-type form, thus representing a biologically motivated model for studying β2-m aggregation.

Materials and Methods *(optional)*

We study the initial stage of the *de novo* aggregation mechanism of D76N by using a three-stage approach based on a plethora of computational tools. We start with I) replica-exchange discrete molecular dynamics (DMD) simulations of a full atomistic native-centric Gō model [11] to identify intermediate states in the folding pathway of D76N. We subsequently employ II) constant-pH molecular dynamics (CpHMD) [12] to investigate up to which extent pH variations may affect the structures of the identified intermediates. By focusing on the dimerization process, which has been reported to be the first stage of β2m fibrillogenesis, we ultimately deploy III) Monte Carlo ensemble docking [11] to determine how the dimerization proceeds for the different intermediates at several pH conditions. We significantly improved this method to construct a cost function based simultaneously **shape, hydrophobic, electrostatic and hydrogen bonding complementarit**y, an evolution in relation to the original cost function exclusively based on shape complementarity.

Results and Discussion *(optional)*

Folding simulations highlight the existence of an aggregation-prone intermediate called I1 which presents an unstructured C-terminus and of an aggregation-prone intermediate featuring two unstructured termini called I2.

N-terminus and C-terminus (mainly at I1) are important players in I1 and I2 dimerization at acidic pH (5.2 and 6.2). DE- and EF-loop are essential in I1 and I2 dimerizational at physiological pH.

Residues Arg-3, Tyr-10 and Arg- 12 in N-terminus, Phe-30 in BC-loop, Phe-56, Trp-60 and Phe-62, Lys-75 in EF-loop and Trp-95 in C-terminus are hotspots in D76N dimerization.

Conclusions *(optional)*

References *(mandatory)*

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