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

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## 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.
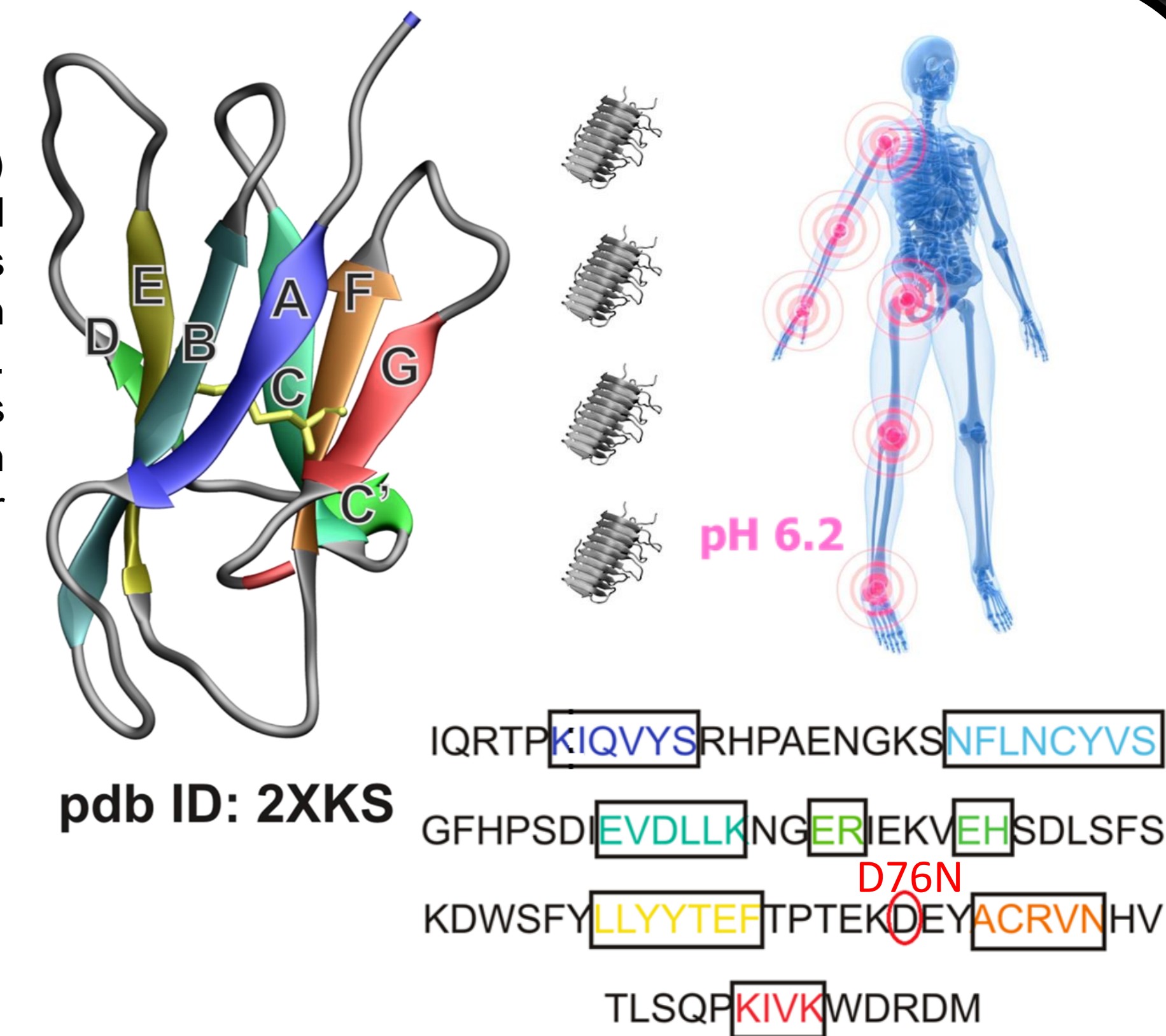
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## Introduction

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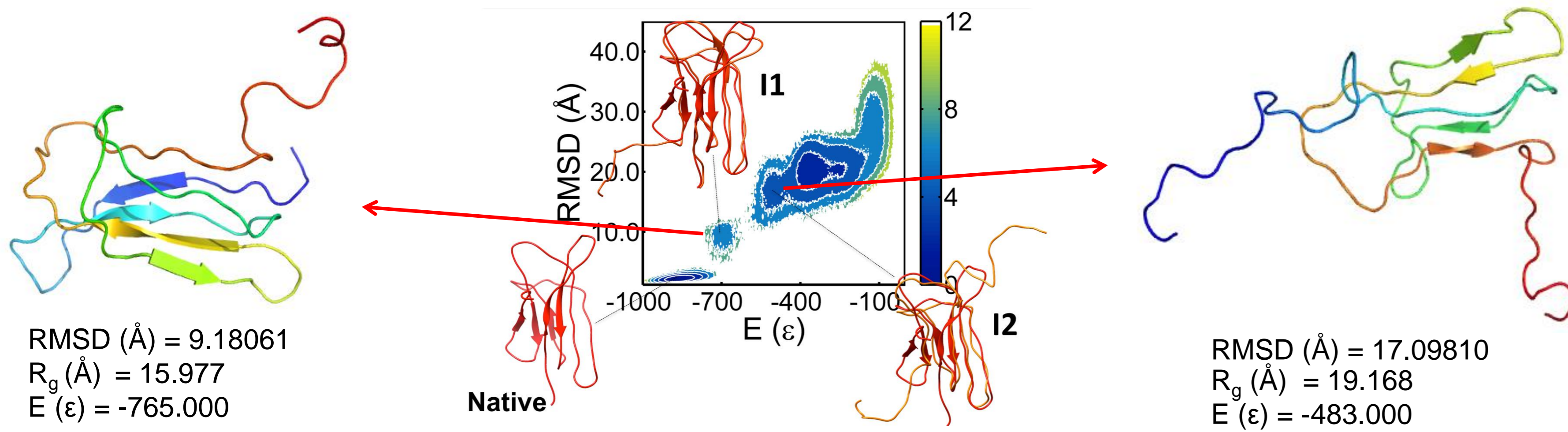
Recently, a single point mutant of β2-m (D76N) was identified as the causing agent of a hereditary systemic amyloidosis affecting visceral organs [4]. This mutant is considerably more amyloidogenic in vitro at physiological conditions than the wild-type form, thus representing a biologically motivated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



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Our goal is to predict the early molecular events of the aggregation mechanism of β2-m using the D76N mutant as a model system. Are there aggregation-prone intermediates populating its folding pathway? What are the main pathways for dimerization? Which residues are critical for the initiation of the aggregation cascade?
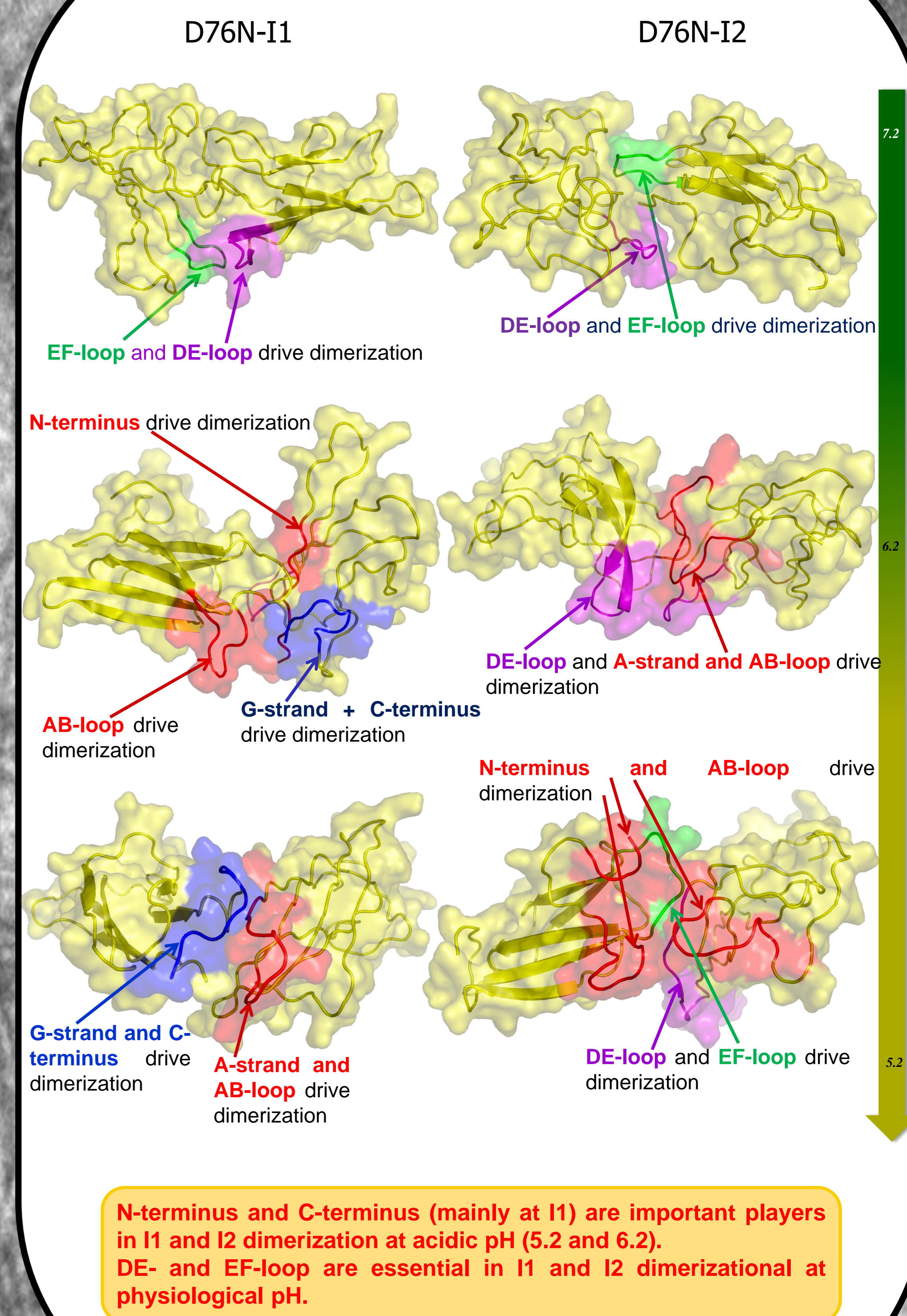
IQRTP KIQVYS RHPAENGKS NFLNCYVS
GFHPSDI EVDLLK NGER IEKV EH SDLSFS
KDWSFY LLYYTEF TPTEK DEY ACRVN HV
TLSQF KIVK WDRDM

## Results

### Folding thermodynamics and intermediate states



RMSD (Å) = 9.18061
Rg (Å) = 15.977
E (ε) = -765.000

The main structural trait of the conformer is an unstructured and detached C-terminus.

RMSD (Å) = 17.09810
Rg (Å) = 19.168
E (ε) = -483.000

The main structural trait of the conformer is unstructured and detached termini (structurally analogous to a recently experimentally identified D76N intermediate).

### Dimerization interfaces
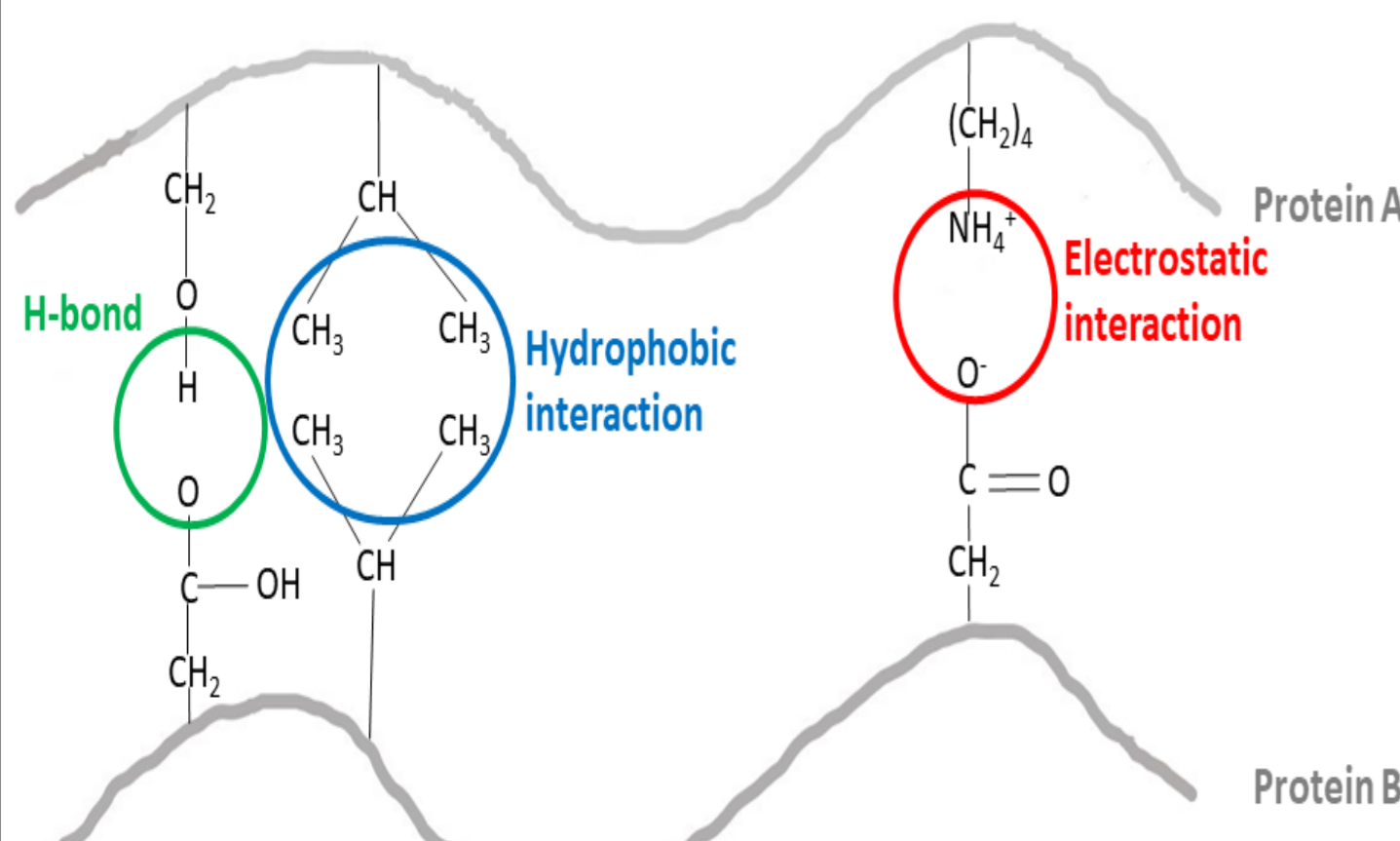


**D76N dimerization hot spots**

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.

**Comparison with experiment**

❖ Phe56, Trp60, Phe62 in the DE-loop are designed dimerization spots because they assist the docking of β2m onto the MHC-I heavy chain [5].

❖ Several experimental studies have also highlighted the role of the above residues of the DE-loop in β2-m oligomerization [6,7,8].

❖ Residue Tyr10 in the strand A [8] have been proposed to be important in β2-m dimerization.

❖ Several reports have provided evidence of the highly dynamic nature of strand A [9,10]. An unstructured strand A implicated in β2m fibrillogenesis has been reported in [8].
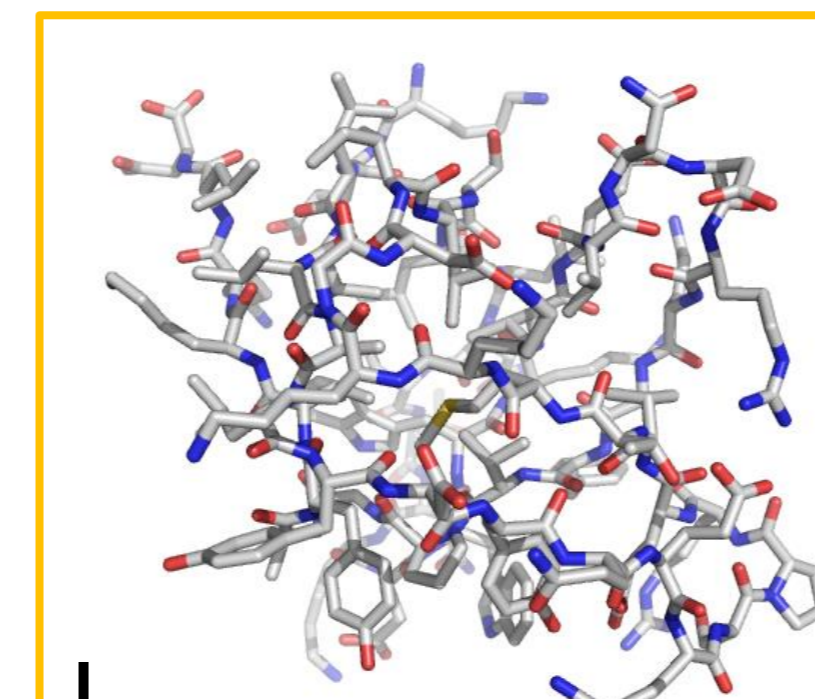
### Dimerization mechanism

D76N-I1                D76N-I2



EF-loop and DE-loop drive dimerization

DE-loop and EF-loop drive dimerization

N-terminus drive dimerization

AB-loop drive dimerization

G-strand + C-terminus drive dimerization

DE-loop and A-strand and AB-loop drive dimerization

N-terminus and AB-loop drive dimerization

G-strand and C-terminus drive dimerization

A-strand and AB-loop drive dimerization

DE-loop and EF-loop drive dimerization

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.
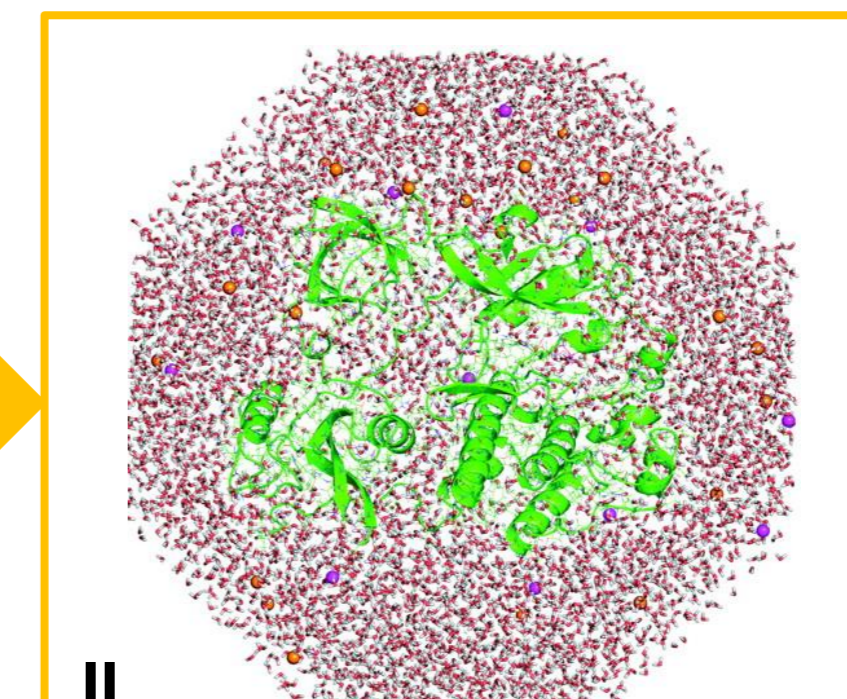
## Methods

Monte Carlo Ensemble Docking



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 on **shape, hydrophobic, electrostatic and hydrogen bonding complementarity**, an evolution in relation to the original cost function exclusively based on shape complementarity.
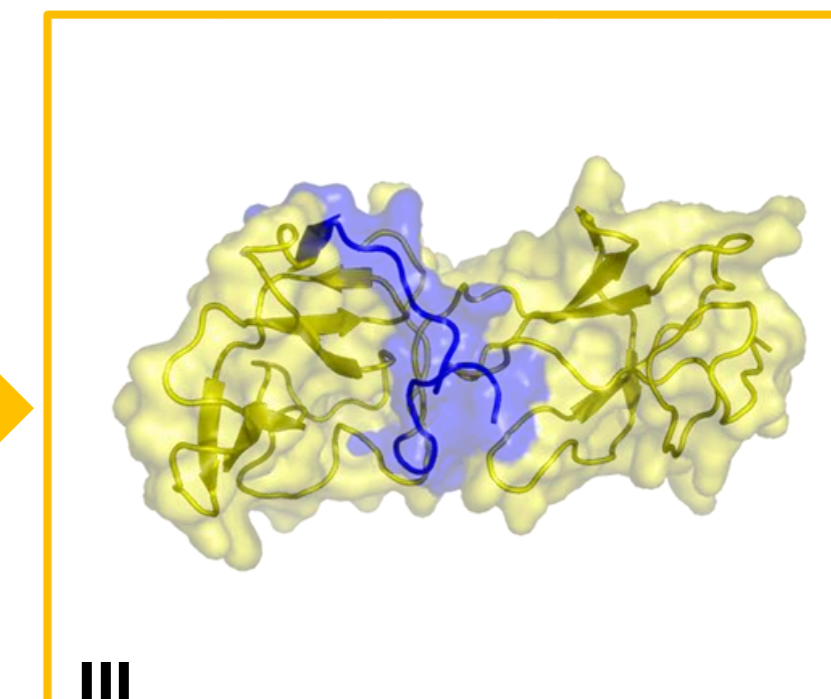
native-centric folding | pH-dependent equilibration | dimerization analysis

(pH 7.2, 6.2, 5.2)

intermediate ensemble extraction and characterization

Monte Carlo ensemble docking

dimer interface analysis
dimer hot spot predictions

## References

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