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# Predicting unfolding thermodynamics and stable intermediates for alanine-rich helical peptides with the aid of coarse-grained molecular simulation

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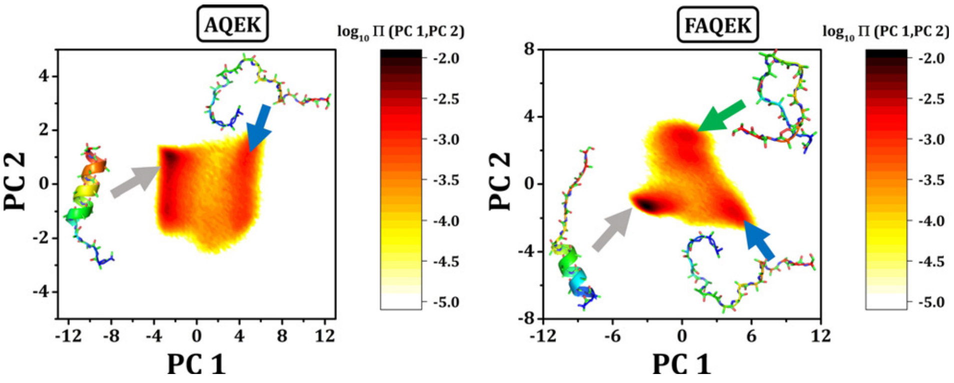
## HIGHLIGHTS

- A 4bAA-CG model predicted helical polypeptide unfolding thermodynamics.
- Unfolding intermediates were mediated by hydrophobic contacts between peptides.
- Inter-peptide interactions modify the unfolding of individual peptides.

## GRAPHICAL ABSTRACT



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## ABSTRACT

This report focuses on the molecular-level processes and thermodynamics of unfolding of a series of helical peptides using a coarse-grained (CG) molecular model. The CG model was refined to capture thermodynamics and structural changes as a function of temperature for a set of published peptide sequences. Circular dichroism spectroscopy (CD) was used to experimentally monitor the temperature-dependent conformational changes and stability of published peptides and new sequences introduced here. The model predictions were quantitatively or semi-quantitatively accurate in all cases. The simulations and CD results showed that, as expected, in most cases the unfolding of helical peptides is well described by a simply 2-state model, and conformational stability increased with increased length of the helices. A notable exception in a 19-residue helix was when two Ala residues were each replaced with Phe. This stabilized a partly unfolded intermediate state via hydrophobic contacts, and also promoted aggregates at higher peptide concentrations.

## 1. Introduction

Unfolding, self-assembly and non-native protein aggregation play important roles in the solution behavior of proteins and polypeptides. In some cases, nonnative aggregation and self-assembly are undesired because they are responsible for the degradation of biopharmaceuticals

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and are linked to a number of debilitating diseases [1–3]. On the other hand, proper protein self-assembly is responsible for much of the physiological functions of proteins *in vivo* [1,4–6].

Additionally, (poly)peptide self-assembly and aggregation can be used as a kinetically controlled and tunable process to form new structures based on various peptide sequences [7–9]. In each case, a molecular-scale description of the process(es) is needed if one wishes to design or predict the behavior and relative stability of key intermediate species – e.g., as a function of peptide sequence and solution environment [7,10–12]. This is experimentally challenging, as few experimental techniques allow for the identification of the role of each specific residue in the unfolding and aggregation behavior of a defined sequence [1,13]. Additionally, such techniques are relatively low throughput, expensive, and/or have large sample material requirements. This poses challenges for testing and design of a range of protein sequences and solution environments, and helps to motivate development of modeling approaches to aid in those efforts [10,14].

With the exception of relatively small proteins and peptides, it is too computationally expensive to simulate most systems of interest with all-atom simulations and explicit solvent molecules [15–18]. Atomistic force fields provide the most accurate method available to identify amino-acid-specific interactions, while coarse-grained (CG) molecular models provide faster computations at the expense of molecular definition [18,19]. Therefore, CG models can potentially be well-suited as a computational framework to speed up experimental searches in order to optimize time and resources, provided the force-field can accurately predict the experimental behavior of interest [18,20,21].

In this regard, a number of 4 beads-per-amino acid (4bAA) CG models have been previously proposed and studied in order to capture the configurational and aggregation behavior of peptides and proteins in solution [7,16,22]. 4bAA models have primarily been designed to capture qualitative structural features of polypeptide unfolding and self-assembly – e.g., the transition between helix and coil configurations for natively helical polypeptides, the existence of local conformations during unfolding, and the formation of tertiary structures for long polypeptides [7,16,22]. Previous work from some of the present authors showed that 4bAA models can help one understand the molecular scale interactions that affect the unfolding and self-assembly of Ala-rich peptides in the context of polymer-peptide interactions [7]. An earlier model was based on that from Bereau and Deserno, which was originally designed to capture structural details of membrane protein assembly [16]. Separately, previous work has shown that the 4bAA level of CG modeling provides an optimal balance between molecular detail, computational accuracy, and computational burden when (poly)peptide unfolding and aggregation are of interest [22].

However, much less effort has been devoted to quantifying the thermodynamics of unfolding and aggregation in CG molecular models. Even though qualitative structural features of the unfolding process are reasonably well captured, the quantitative details such as unfolding free energy values, midpoint unfolding temperatures ($T_m$), and unfolding enthalpy values obtained from molecular simulations (both CG and atomistic) typically do not match those obtained experimentally [7,16,22–25]. Furthermore, it is common to use molecular simulations in a “hindsight” manner, where the experimental behavior is already known and the simulations are intended to give molecular-scale insight that is beyond the capabilities of the experiment, or to help confirm or refute hypotheses that were based on interpretation of the experimental data. Much less work has been devoted to predicting experimental behavior *a priori* with CG models.

This motivates the first part of the present work, which is focused on refining the previously proposed 4bAA protein model [7,16] to more accurately quantify unfolding thermodynamics for a series of helical Ala-rich polypeptides as a function of chain length. From there, the model was used with Replica Exchange Molecular Dynamics to make *a priori* predictions for the unfolding thermodynamics and pathways for a set of new Ala-rich peptides that were then experimentally synthesized and characterized with circular dichroism spectroscopy, for comparison to model predictions. The particular choices of peptides were based on previous Ala-rich sequences and future applications that focus on control of peptide-peptide interactions in multiblock peptide-polymer conjugates to manipulate assembly [8,9,26–33]. The simulated and experimental unfolding thermodynamics were in quantitative or semi-quantitative agreement across the range of peptides tested, but the model also predicted outliers compared to the expected peptide behavior that was based on qualitative reasoning regarding amino acid hydrophobicity and the length of the peptide chain. In that case, stable folding intermediates were responsible for non-2-state behavior, and the CG simulations suggested key intra-molecular interactions that stabilized partly folded intermediate structures and complicated the unfolding transitions of even these relatively small, Ala-rich helices. Inter-peptide interactions were also shown to stabilize partly unfolding conformers that may be important as intermediates for larger-scale aggregation, consistent with preliminary experimental results.

## 2. Material and methods

### 2.1. Peptide synthesis and purification

All materials were purchased from Fischer Scientific (Pittsburgh, PA) except where otherwise indicated. Peptides were synthesized on a Rink Amide Resin (ChemPep, Wellington, FL). Specifically, the sequences listed in Table 1 (shorthand notations used in Table 1: AQEK, FAQEK, and AQK18) were synthesized with a PS3 peptide synthesizer (Protein Technologies, Tucson, AZ). Longer sequences (shorthand notation in Table 1: AQK27 and AQK35) were synthesized with a Focus XC peptide synthesizer (AAPTec Inc., Louisville, KY). Fmoc-alanine, Fmoc-lysine(boc), Fmoc-glutamic acid (t-butyl), Fmoc-glutamine(trt), and Fmoc-phenylalanine were all purchased from ChemPep. The N-terminus of each peptide was acetylated, and peptides were cleaved in 95% trifluoracetic acid (TFA), 2.5% $H_2O$, and 2.5% triisopropylsilane (Sigma-Aldrich, St. Louis, MO). TFA was mostly evaporated, and peptides were then precipitated twice into cold ethyl ether. Samples were redissolved in water, frozen in liquid nitrogen, and lyophilized. Dried samples were then reconstituted in water and purified by preparative-scale reverse-phase high-performance liquid chromatography (RP-HPLC) using a Waters Xbridge BEH130 Prep C-18 column. The mobile-phase comprised gradients of degassed, deionized water with 0.1% TFA and acetonitrile with 0.1% TFA, at a flow rate of 21 ml/min. Peptide was detected by UV absorbance at 214 nm, and fractions were collected and lyophilized. Molecular weights of the purified peptides were verified by electrospray ionization mass spectroscopy (ESI-MS).

### 2.2. Circular dichroism spectroscopy (CD)

Experimental characterization of the average secondary structure of peptide samples was conducted via circular dichroism spectroscopy on a Jasco 810 circular dichroism spectropolarimeter (Jasco Inc., Easton, MD, USA). Peptides were dissolved in 10 mM potassium phosphate at pH 7.4 with a final peptide concentration of 0.125 mg/ml. Ionic strength was adjusted for select sample preparation by addition of 500 mM potassium chloride or potassium fluoride stock solutions. Samples were briefly sonicated to aid in the dissolution of the lyophilized peptides,

**Table 1**
Synthesized peptide sequences and short-hand notations.

| Sequence | Short-hand notation | Molecular weight (kDa) |
|---|---|---|
| AAQEAAAAQKAAAAQEAAA | AQEK | 2.04 |
| AAQEFAAAQKAAAFQEAAA | FAQEK | 2.19 |
| $K(AAAQ)_4K$ | AQK18 | 1.95 |
| $K(AAAQ)_3K(AAAQ)_3K$ | AQK27 | 2.92 |
| $K(AAAQ)_4K(AAAQ)_4K$ | AQK35 | 3.75 |