

# Oxidative Surface Mapping Enables Experimental Evaluation of Molecular Dynamics Simulations: An Integrated Strategy for Studying the Tanford Transition in b-Lactoglobulin

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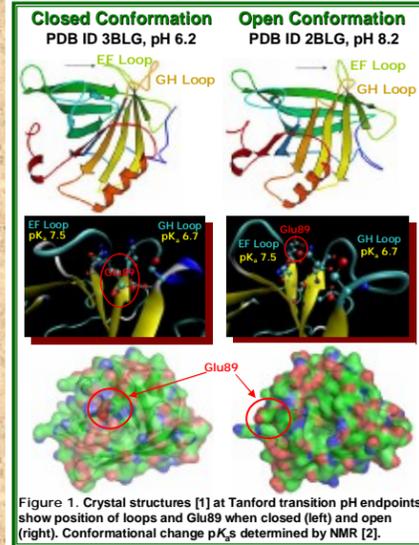


## OVERVIEW

- High resolution structural data is often unavailable for a given protein, or for multiple conformations of a resolved protein structure
- Computational models can be generated for unknown structures by protein fold prediction algorithms that consider:
  - Sequence homology to known domains or protein families
  - Docked ligand-protein or protein-protein interactions
- Simulation of protein behavior *in silico* can provide insight that experiments alone may not, but results require experimental validation
- Goal is to use molecular dynamics (MD) to simulate an increasing pH-induced conformational change, and monitor this change experimentally by probing solvent accessibility (SA) in native fold
- Analysis of covalently labeled peptides by mass spectrometry (MS) and comparison with MD results fosters an integrated approach that may yield clues to protein function

## INTRODUCTION

- b-lactoglobulin A is a cargo protein that is abundant in milk (lipocalin family)
  - Binds fatty acids, hormones, etc.
- Tanford transition involves a pH-sensitive conformational change to expose hydrophobic pocket
  - EF loop "lid" opens around pH 7-7.5
  - Glu89 pK<sub>a</sub> shifted from 5 to 7.5



## EXPERIMENTAL

### Model Building for Molecular Dynamics

- Initial Coordinates and Modifications**
  - Two models starting with 3BLG structure at pH 6.2
  - Hydrogens built onto heavy atoms in XRC structure; all titratable residues were ionized, including some His
  - Control model remains protonated at Glu89, experimental model is deprotonated (ionized) at Glu89
- Implicit Solvation**
  - Effective dielectric constant without H<sub>2</sub>O molecules
- Net Charge Neutralization**
  - Replaced some H<sub>2</sub>O with Na<sup>+</sup> to achieve zero net charge
- Energy Minimization**
  - Bulk solvent was energy minimized after fixing all bonds involving hydrogens
- Heating**
  - Thermodynamic annealing over 10 ps prepares the system for dynamics
- Molecular Dynamics**
  - Dynamics were performed at 298 K; trajectory files generated in 200 ps increments with 1000 frames each

### Oxidative Surface Mapping Protocol

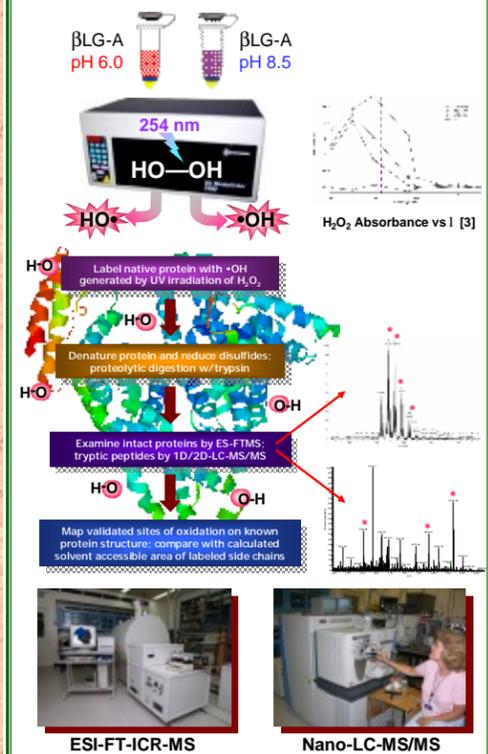
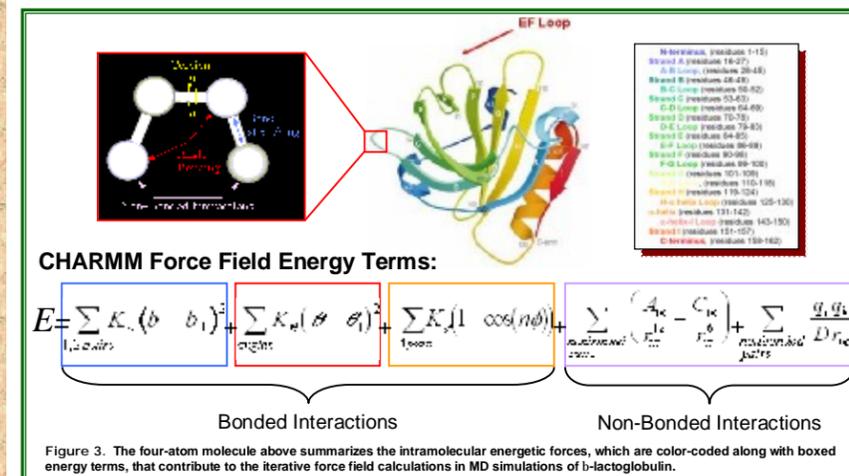


Figure 2. Hydroxyl radicals are generated by ultraviolet (UV) photolysis of hydrogen peroxide; these short-lived reactive oxygen species react with thiol, aromatic, and some aliphatic groups on solvent accessible amino acids [4]. Reactions occurred in pH-controlled (6 or 8.5) solutions under UV lamp, then were quenched by SepPak (Waters) extraction for MS analysis. Oxidation depth on intact proteins measured by direct infusion ESI-FT-ICR-MS (Varian) and peptides measured by reverse phase liquid chromatography/tandem mass spectrometry on LTQ-Orbitrap (Thermo) for high mass accuracy to determine oxidative modifications. (Orbitrap photo picturing P. Lankford taken by G. Hurst)

## MOLECULAR DYNAMICS SIMULATIONS

### Basics of Molecular Dynamics (MD) Simulations:

- Build two models from known structure (pH 6.2 structure PDB 3BLG)
- Maintain control model and perturb structure of experimental model → Deprotonate key Glu89 residue
- Observe structural conformational adjustments as energy states are randomly sampled from state A (closed) to state B (hypothesized to be open for experimental model, while remaining closed in control model)
- Examine time-points for positional changes of structural elements; correlate these with free energy changes



### Results from Molecular Dynamics Simulations

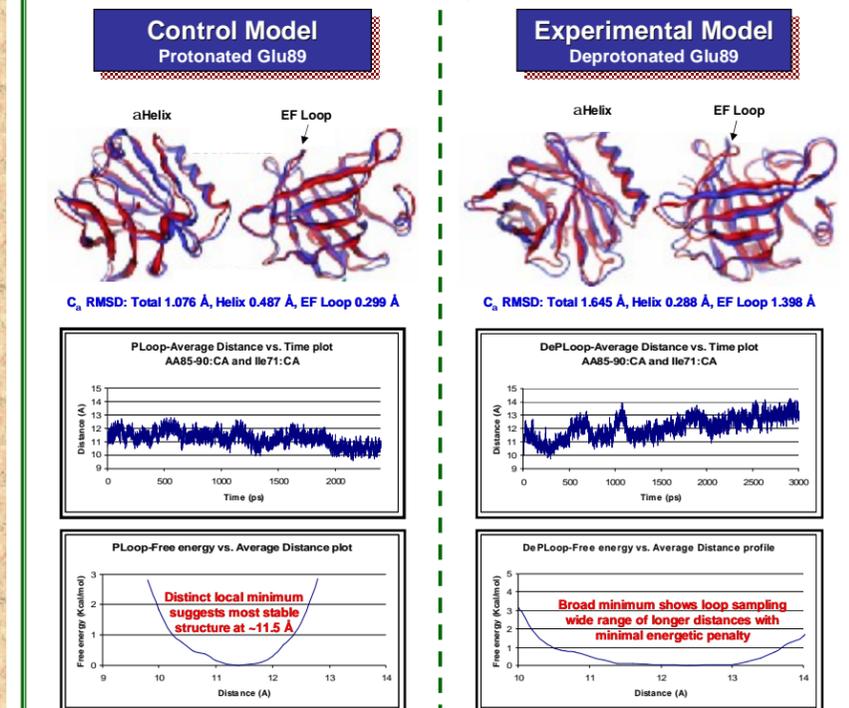
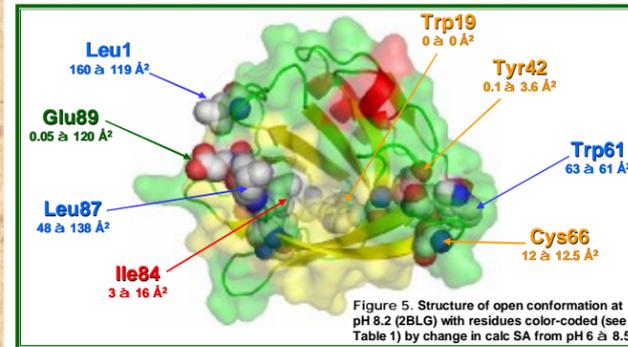


Figure 4. Structures show front and back views of overlaid backbone ribbons for starting state A (red) and state B (blue) after 2.5 or 3 nanoseconds, for the protonated control or deprotonated experimental models, respectively; degree of structural similarity reported by global and local root mean square differences (RMSD) among C<sub>α</sub> atoms in backbones. Graphs calculated by tracking average distance of six loop residues from cavity rim residue Ile71 over MD timeframe and binning free energy of the system by loop distance.

## SURFACE MAPPING DATA



### Amino Acid Oxidations

- Residues oxidized in denatured control sample listed in left column
- Percentages reflect ratio of oxidized peptide to total (normal + oxidized) identified under each condition
- Only the highest scoring matches were counted; isomers in a single MS/MS not considered (see Figure 6)
- Data not in agreement with SA may be due to dynamic SA in solution (B-factor), reactivity affected by neighbors (low % in denatured), or uncounted isomers \*

Residue	pH 6	pH 8.5	Denature
Leu1	3%	7%	24%
Met2	1%	1%	2%
Met7	64%	84%	56%
Leu10	-	-	8%
Trp19	-	-	6%
Trp20	-	-	8%
Met24	39%	51%	63%
Tyr42	-	-	7%
Pro58	-	-	7%
Leu54	-	-	7%
Trp61	2%	2%	100%
Cys66	-	-	100%
Phe82	9%	-	21%
Ile84	-	-	24%
Leu87	4%	4%	2%
Tyr99	-	-	2%
Pro126	1%	1%	1%
Leu133	17%	14%	15%
Leu143	17%	-	17%
Pro144	21%	-	17%
Met145	50%	50%	100%
Leu149	-	-	25%

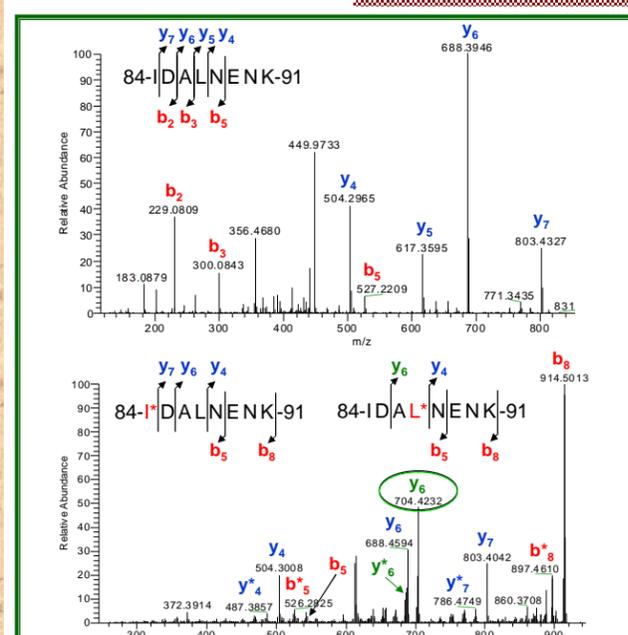


Figure 6. Tandem mass spectra from pH 8.5 sample of oxidized BLG showing peptide 84-91, which spans portions of beta-strands E and F and encompasses the EF loop. Top: Mass spectrum of normal (unoxidized) peptide. Bottom: Mass spectrum of oxidized peptide illustrating co-eluting mono-oxidized isomers, which present an analytical challenge for data interpretation.

## CONCLUSIONS

- b-lactoglobulin provides a good model system for evaluating conformational changes by computational & experimental methods
- MD predicted EF loop movement away from cavity, suggesting start of the pH-sensitive Tanford transition
  - Many energy states are sampled over dynamics timeframe (3 ns)
  - Longer MD simulation to mirror timescale of conformational change (ms to ms) may yield more complete transition
- Oxidative surface mapping confirmed solvent accessibility status for several residues during the cavity opening
  - The hydroxyl radical lifetime is on the order of nanoseconds and it targets many different residues
  - Currently limited in extraction of oxidation data due to isomers and potential for complex products
  - Oxidative data could be confirmed and complemented by additional labeling techniques in parallel
- At present, molecular dynamics and surface mapping results have to be compared manually, but should be better integrated to strengthen each approach

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