

# Computer Simulations of Protein Refolding and Aggregation in the Presence of Solutes

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**Objective:** to perform computer simulations that to examine the mechanisms by which the addition of solutes affects the competition between protein refolding and aggregation.  
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## Introduction:

- Recombinant proteins frequently agglomerate to form insoluble particles termed inclusion bodies.
- To recover active protein, the aggregates have to be solubilised first by exposure to a strong denaturant, and then the resultant de-aggregated proteins have to be refolded via renaturation.

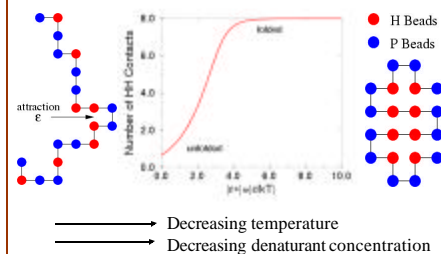


- Adding certain solutes to the solution during the renaturation step efficiently reduces aggregation and thus increases the refolding yield.  
→ An understanding of the molecular mechanism underlying the competition between refolding and aggregation in the presence of solutes is important.

## Method:

### Protein Representation: HP Model (Lau and Dill, 1989)

- A protein molecule is represented as a sequence of hydrophobic **H** and polar **P** residues called "beads" on a two-dimensional square lattice.
- Hydrophobic effect: non-bonded neighboring H beads attract each other with energy  $\epsilon$ , a measure of denaturant concentration or temperature.



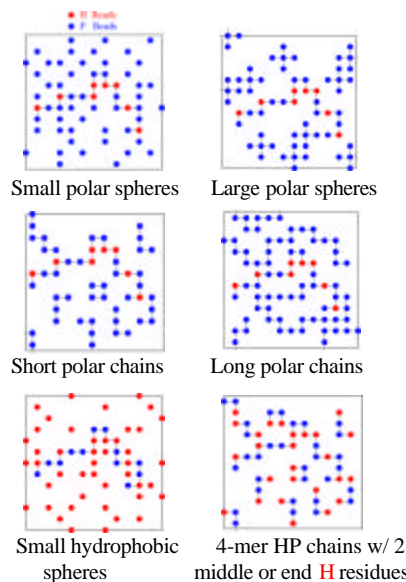
### Algorithm: Dynamic Monte Carlo

- Randomly perturb a small section of the chain to create a new configuration
- If the new configuration results in a lower overall energy, accept the perturbation; otherwise, accept it according to the Metropolis acceptance criteria

### Modeled Parameters:

- Denaturant concentration or temperature:  $|\epsilon^*| = \epsilon/kT$
- The hydrophobicity of solute with protein:  $\epsilon_{SP}$
- Protein concentration (packing fraction)  $\rho_{\text{protein}}$
- Solute concentration (packing fraction)  $\rho_{\text{solute}}$

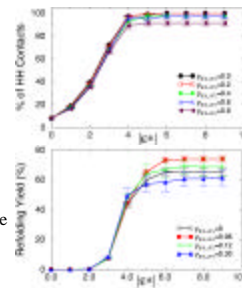
## Solute Models



## Results:

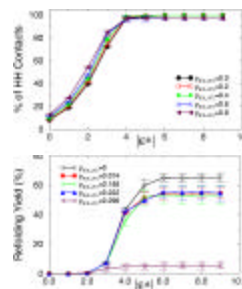
### Small Polar Solutes

- The presence of small polar solutes do not affect the stability of isolate proteins.
- In multi-protein systems ( $\rho_{\text{protein}} = .60$ ), the refolding yield is slightly improved by the presence of small polar solutes at low solute concentrations.



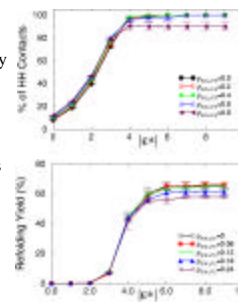
### Large Polar Solutes

- The presence of large polar solutes do not affect the stability of isolate proteins.
- In multi-protein systems ( $\rho_{\text{protein}} = .60$ ), the refolding yield is slightly reduced by the presence of small polar solutes at all solute concentrations.



## Short Chain-like Polar Solutes

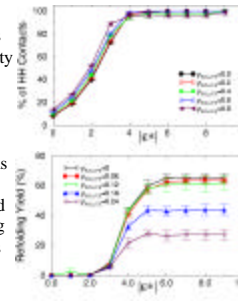
- The presence of short chain-like polar solutes do not affect the stability of isolate proteins unless at high solute concentrations.



- In multi-protein systems ( $\rho_{\text{protein}} = .60$ ), the yield is slightly reduced by the presence of short chain-like polar solutes at high concentrations.

## Long Chain-like Polar Solutes

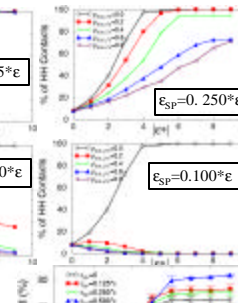
- The presence of long chain-like polar solutes do not affect the stability of isolate proteins at all solute concentrations.



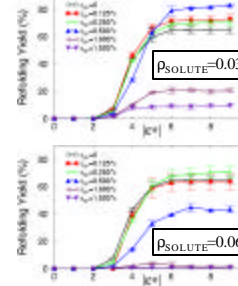
- In multi-protein systems ( $\rho_{\text{protein}} = .60$ ), the yield is significantly reduced by the presence of long chain-like polar solutes at high concentrations.

## Small Hydrophobic Solutes

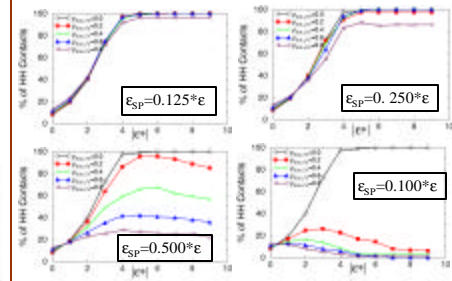
- The presence of small hydrophobic solutes affect the stability of isolate proteins at all solute concentrations.
- In multi-protein systems ( $\rho_{\text{protein}} = .60$ ), the yield is slightly improved by the presence of small hydrophobic solutes at low concentrations.



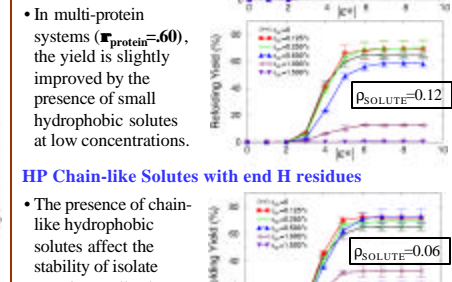
- The presence of small hydrophobic solutes affect the stability of isolate proteins at all solute concentrations.
- In multi-protein systems ( $\rho_{\text{protein}} = .60$ ), the yield is slightly improved by the presence of small hydrophobic solutes at low concentrations.



## HP Chain-like Solutes with middle H residues

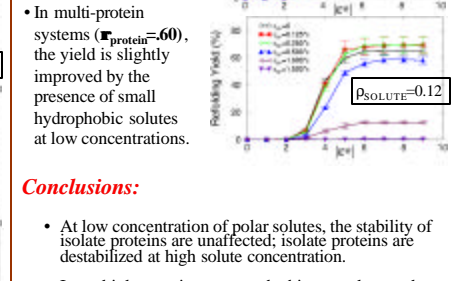


- The presence of chain-like hydrophobic solutes affect the stability of isolate proteins at all solute concentrations.
- In multi-protein systems ( $\rho_{\text{protein}} = .60$ ), the yield is slightly improved by the presence of small hydrophobic solutes at low concentrations.



## HP Chain-like Solutes with end H residues

- The presence of chain-like hydrophobic solutes affect the stability of isolate proteins at all solute concentrations.
- In multi-protein systems ( $\rho_{\text{protein}} = .60$ ), the yield is slightly improved by the presence of small hydrophobic solutes at low concentrations.



## Conclusions:

- At low concentration of polar solutes, the stability of isolate proteins are unaffected; isolate proteins are destabilized at high solute concentration.
- In multiple-protein systems, the bigger or longer the solutes are, the more the refolding yield is reduced especially at high concentration of polar solutes.
- Small hydrophobic solutes destabilize isolate proteins more significantly than chain-like solutes.
- When the strength of the solute-protein interaction is weak, the presence of small interacting solutes and chains slightly improves the refolding yield at low solute concentrations.
- When the strength of the solute-protein interaction is strong, the presence of any type of interacting solutes decreases the refolding yield.