

Computer Simulations of Protein Aggregation

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Introduction

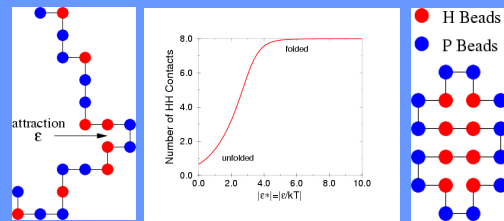
- Recombinant proteins frequently agglomerate to form insoluble particles termed inclusion bodies.
- To recover active protein, the aggregates have to be solubilised first by exposure to a strong denaturant, and then the resultant de-aggregated proteins have to be refolded via renaturation.
- Renaturation is done via dilution, dialysis, dialfiltration or pulse renaturation methods.
- During the refolding process, the unfolded chains begin to re-aggregate resulting in low yields of active protein.



→ An understanding of the molecular mechanism underlying the competition between refolding and aggregation is important

HP Protein 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 ϵ , a measure of denaturant concentration or temperature.



→ Decreasing temperature
 → Decreasing denaturant concentration

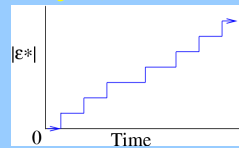
Computational Algorithm: Dynamic Monte Carlo

- 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

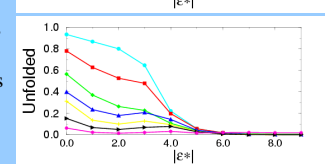
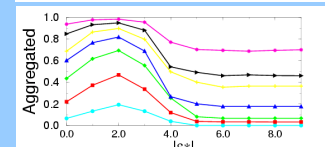
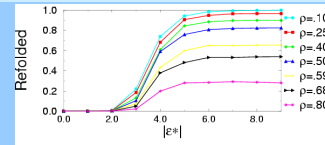
Modeled Parameters:

- Denaturant concentration or temperature: ϵ/kT
- Protein concentration (packing fraction): ρ

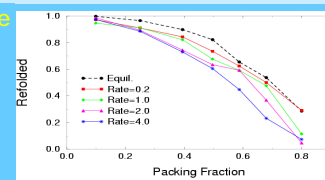
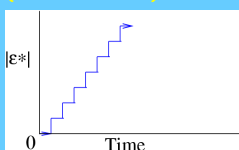
Infinitely Slow Cooling (Dialysis)



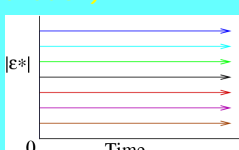
- Refolding yield depends on packing fraction
- All systems remain stable after $|\epsilon^*| = 6.0$
- The fraction aggregated exhibits a maximum at $|\epsilon^*| = 2.0$
- Small aggregates: mostly dimers and trimers at low packing fraction



Slow Cooling at Finite Rate (Dialfiltration)



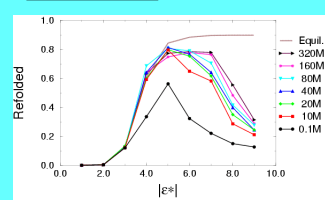
Quenching (One-step dilution)



- Extremely long time to reach equilibrium; the maximum eventually disappears, in agreement with experiments (De Bernardes-Clark & others, 1997)
- Aggregates are large

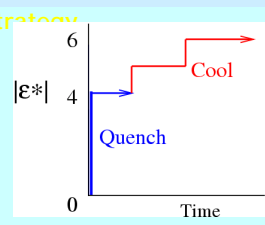
- Refolding yields depend on the cooling rate
- Similar refolding yields as in the infinitely slow cooling case but 10 times quicker

Refolding yield at $\rho = .40$ obtained at different times:



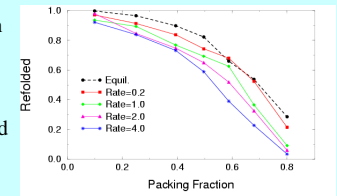
Suggested Quenching: equilibrium Strategy

- Based on:
- can be reached quickly at $|\epsilon^*| = 4.0$
 - Slow but finite cooling: slower cooling produces higher refolding yields
 - Infinitely slow cooling: cooling further than $|\epsilon^*| = 6.0$ is not useful

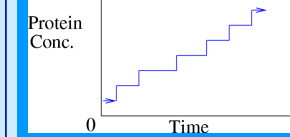


Results from Suggested Strategy

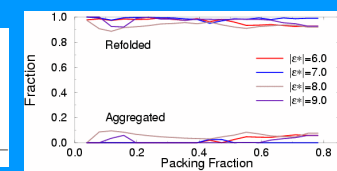
- Similar refolding yields as in the slow but finite cooling case but 3 times quicker
- High refolding yield obtained in short time



Pulse Renaturation (Fed-batch Operation)

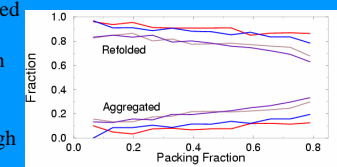


1 chain added per pulse:



- Starting configurations are at low packing fraction
- Stepwise addition of denatured chains: 1, 2, or 5 chains
- New chains added when then system reached equilibrium
- Refolding yields depend on addition rate and are very high

5 chains added per pulse:



Conclusions

- We employed a low-resolution model to simulate four renaturation methods. We learned that:
 - The maximum that appears in quenching disappears after a long time
 - Slow cooling is more effective than quenching: higher yield, shorter time
 - Slow cooling gives smaller size aggregates than quenching
 - Pulse renaturation produces the highest refolding yield even at high packing fractions
- We plan to
 - Study refolding and aggregation in the presence of solutes using the 2-d lattice model
 - Study refolding and aggregation using the 3-d lattice model with side chains
 - Study the formation of ordered aggregates such as fibrils and prions using an off-lattice intermediate-resolution protein model that is designed for use with the discontinuous molecular dynamics (DMD) algorithm

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