Computer Simulation Studies of Protein Folding and Aggregation Using Lattice Models

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Motivation

- Protein aggregation is implicated in seventeen protein deposition diseases including Alzheimer's, Huntington's, Parkinson's, sickle cell disease, Mad Cow disease, cataracts, etc.

- Protein aggregation is a serious problem in biotechnology because it can interfere with the recovery of active protein.

Objectives

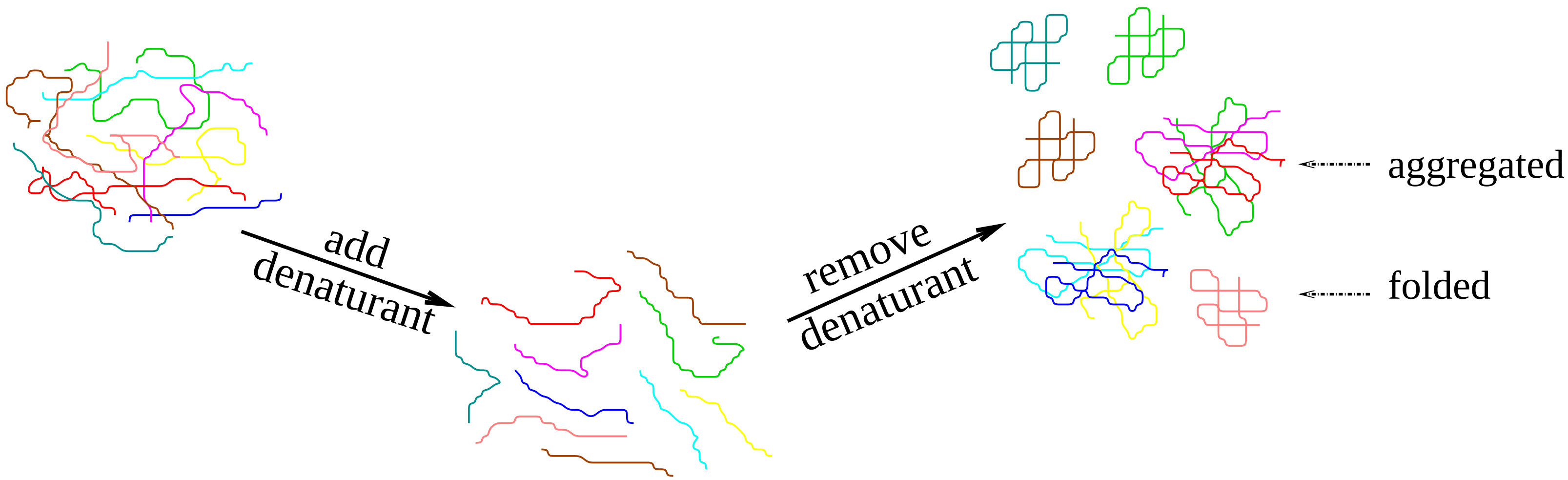
- To study the competition between refolding and aggregation of proteins

- To study how protein refolding and aggregation kinetics are affected by the rate of varying:

  - denaturant concentration
  - temperature
  - protein concentration

Aggregation Interferes with Protein Renaturation

- Renaturation is the most critical and complicated step in recovering native protein from inclusion bodies



- Renaturation is done via dilution, dialysis, dialfiltration or pulse renaturation methods

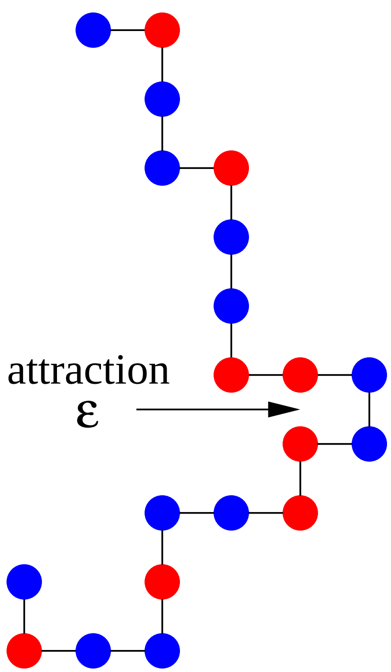
Literature Review: Renaturation of Hen Egg-White Lysozyme (HEWL)

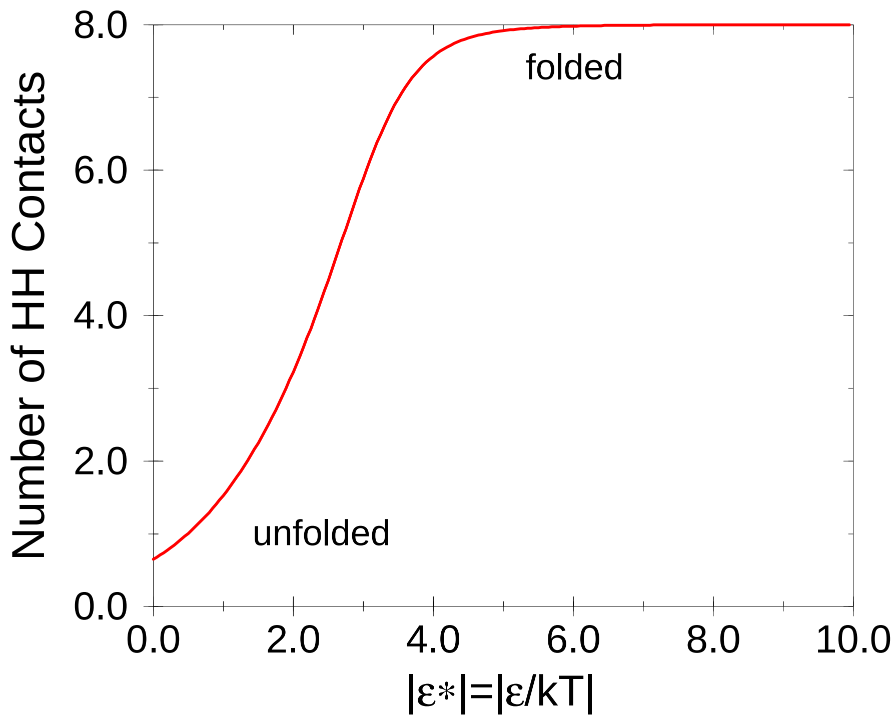
- HEWL is a small well-studied disulphide-bonded protein, refolded by oxidative renaturation

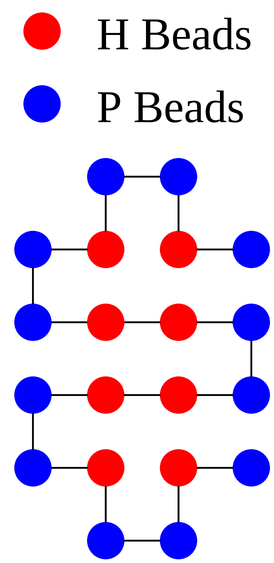
Refolding method	Conc. (mg/mL)	Time (hr)	Yield (%)	Reference
Dilution	5	24	82	Hevehan *et al.*, ’97
Dialysis	5	66	82	Maeda *et al.*, ’95
Diafiltration	5	11	76	Yoshii *et al.*, ’00
Fed-batch	3	24	80	Katoh *et al.*, ’00

Previous Work: Gupta & Hall (1997)

H-P model with the hydrophobic effect (Lau and Dill, 1989)





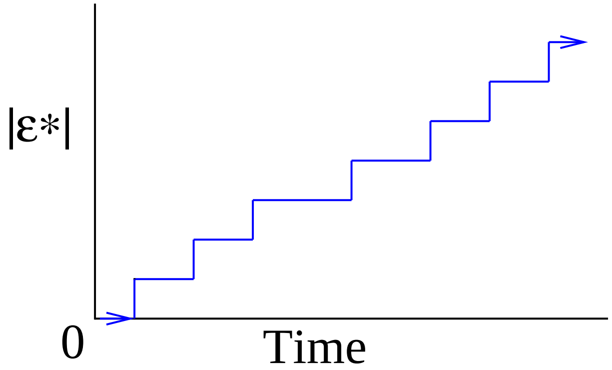


$\longrightarrow$ Decreasing temperature

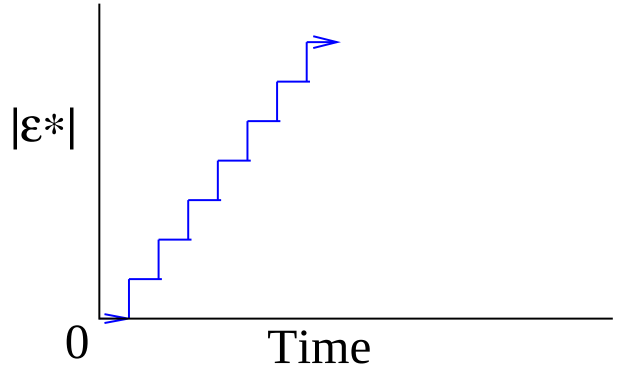
$\longrightarrow$ Decreasing denaturant concentration

Simulations of Four Renaturation Methods

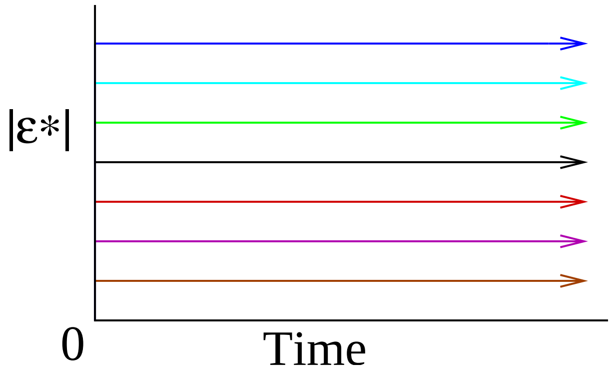
- Infinitely slow cooling (dialysis)



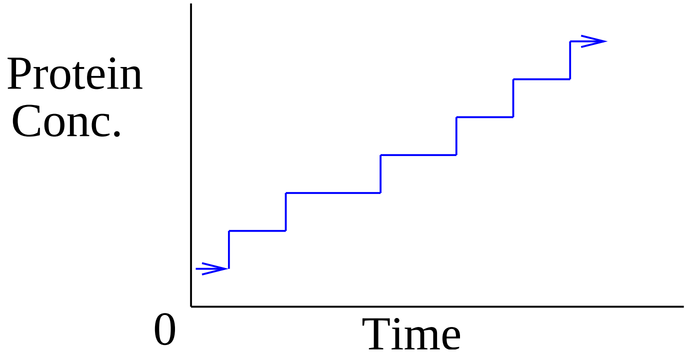
- Slow cooling at finite rate (diafiltration)



- Quenching (one-step dilution)

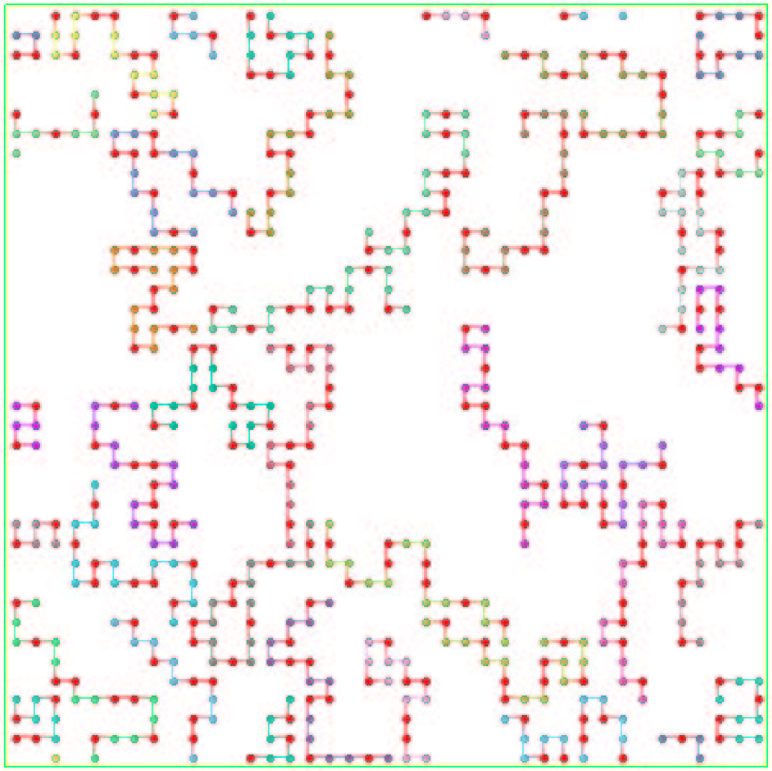


- Pulse renaturation (fed-batch operation)

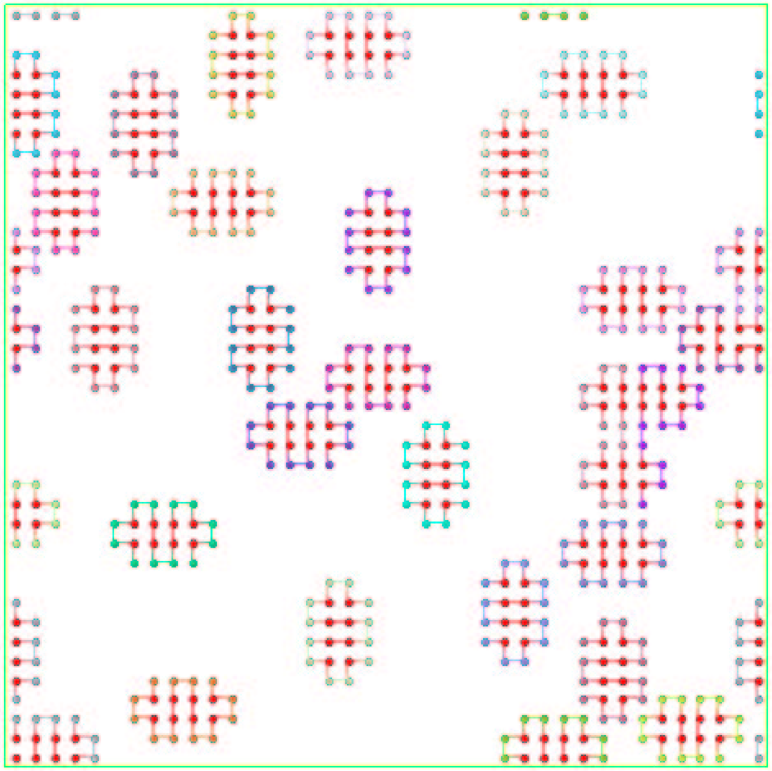


Simulation Method: Dynamic Monte Carlo

At the denatured state:

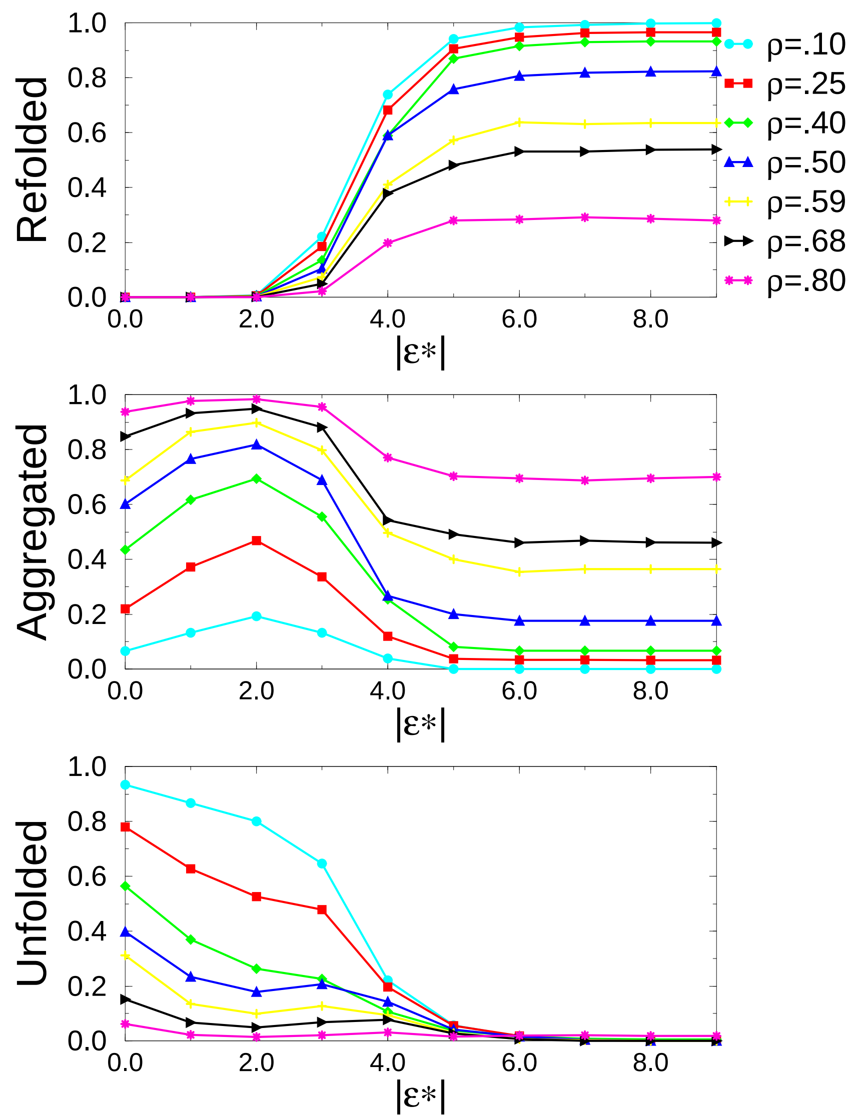


At equilibrium:



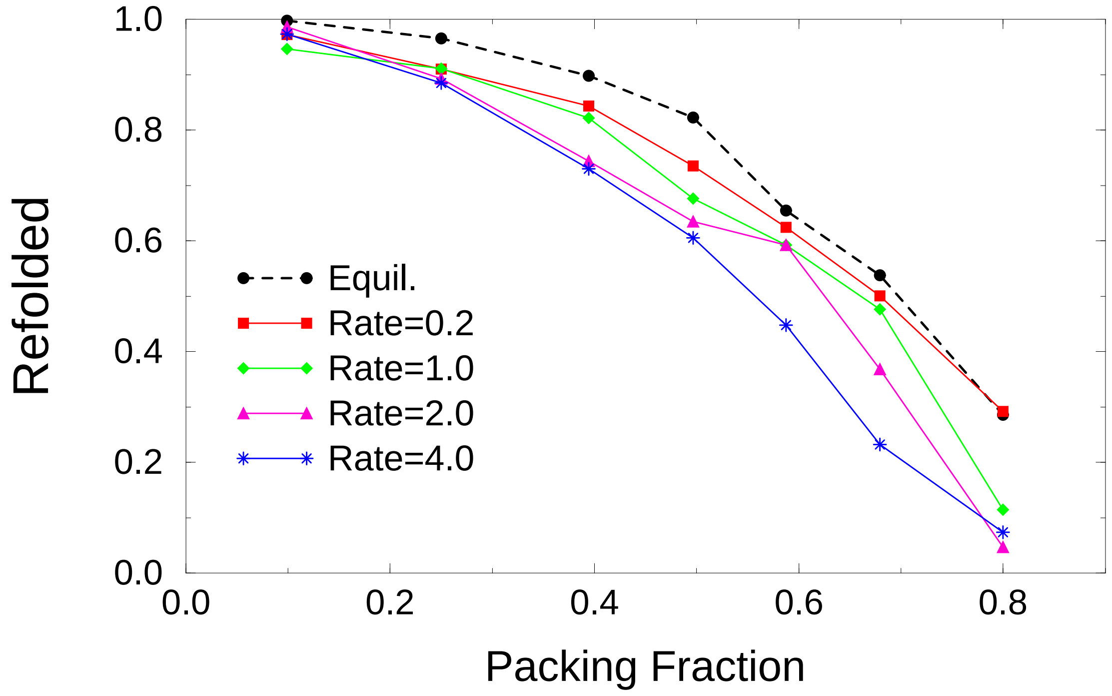
Infinitely Slow Cooling

- 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 at low packing fraction, dimers and trimers at higher packing fraction



Slow Cooling at Finite Rates

- Fix the $|\epsilon^*|$ increment at 1.0, simulate for 0.25,0.50,1.00 or 5.00 million timesteps at each cooling step

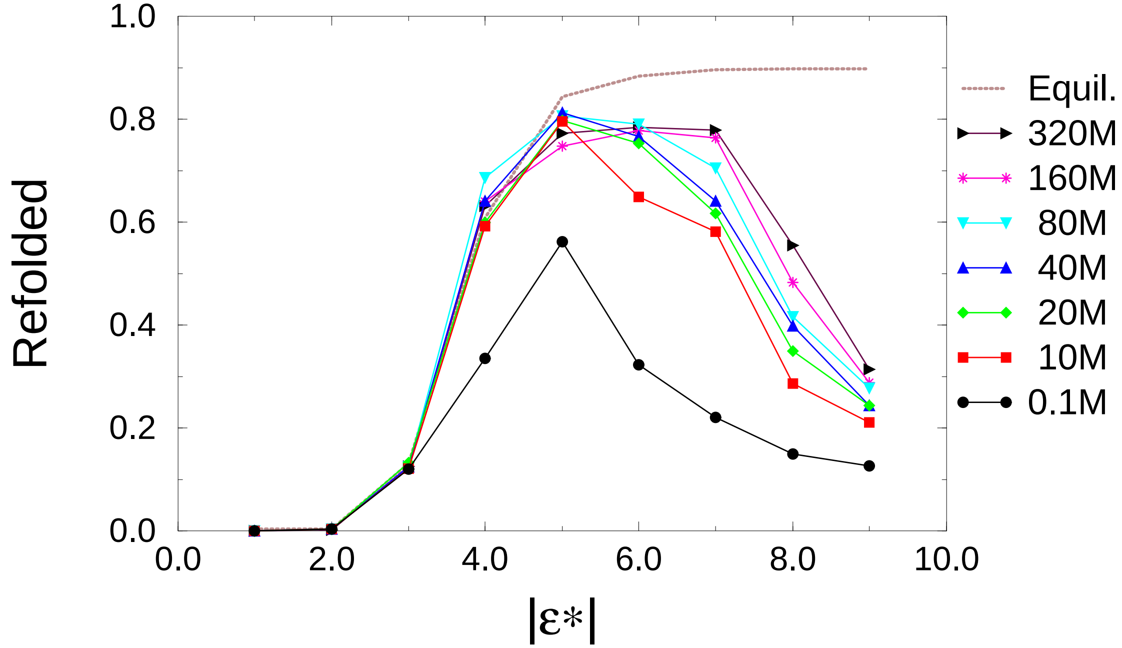


- Refolding yields depend on the cooling rate

- Similar refolding yields as in the infinitely slow cooling case but 10 times quicker

Quenching

- Refolding yield at packing fraction = .40 obtained at different times:



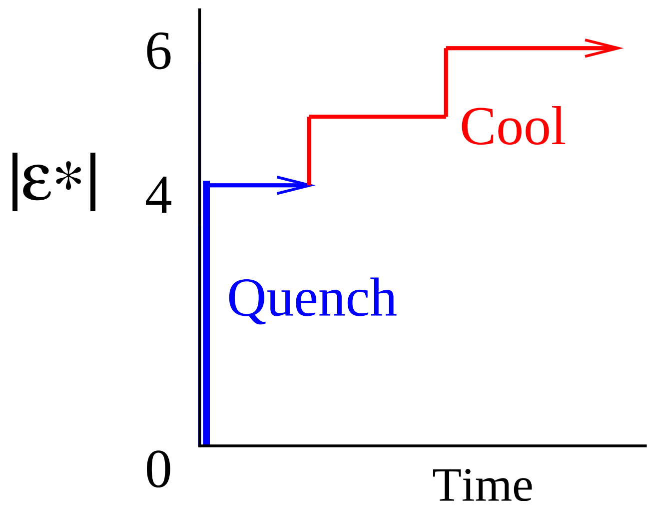
- Extremely long time to reach equilibrium; the maximum eventually disappears, in agreement with experiments (De Bernardez-Clark *et al.*, 1997)

- Aggregates are large

Suggested Strategy to Obtain High Refolding Yield in Short Time

Based on:

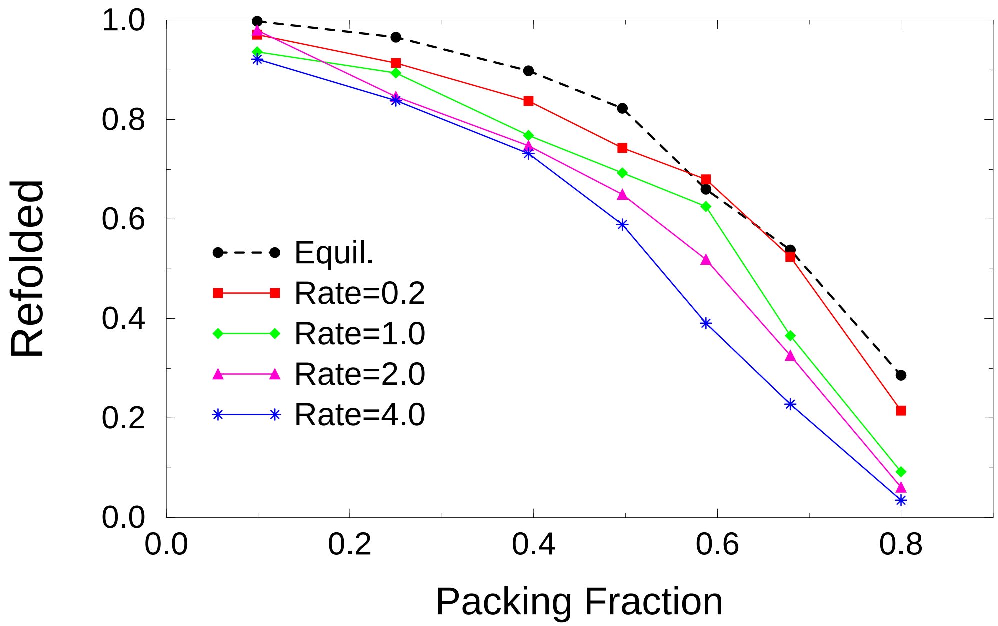
- Quenching: equilibrium can be reached quickly at $|\epsilon^*| = 4.0$
- Slow but finite cooling: slower cooling produces higher refolding yields
- Infinitely slow cooling: cooling further than $|\epsilon^*| = 6.0$ is not useful



Results from the Suggested Strategy

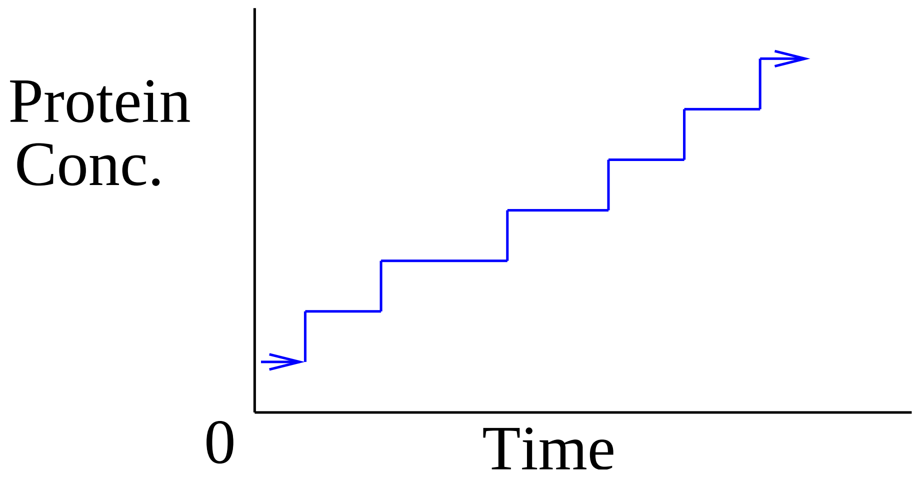
- Similar refolding yields as in the slow but finite cooling case but 3 times quicker

- High refolding yield obtained in short time



Pulse Renaturation

- Starting configurations are at low packing fraction

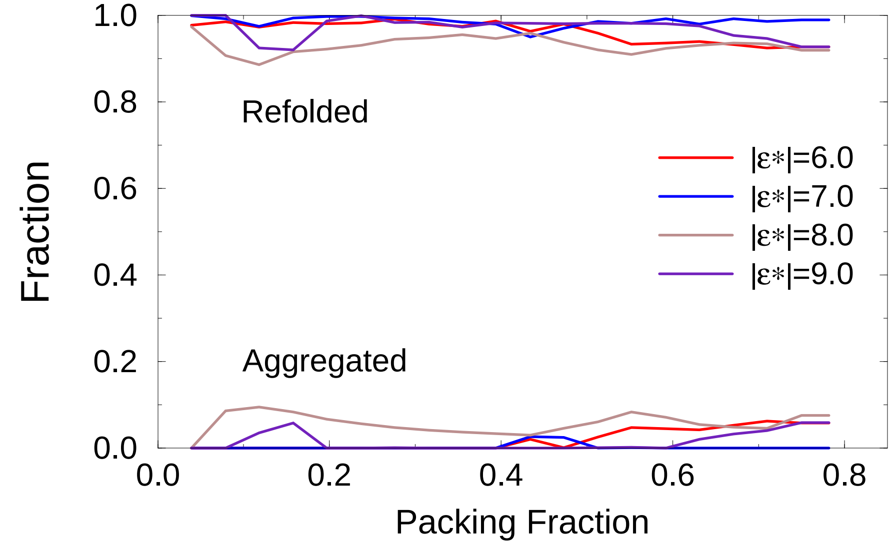


- Stepwise addition of denatured chains: 1, 2, or 5 chains
- New chains added when refolding yield, fraction aggregated, and total number of HH contacts become constant
- Runs conducted at $|\epsilon^*|=$ 6.0, 7.0, 8.0, and 9.0

Pulse Renaturation

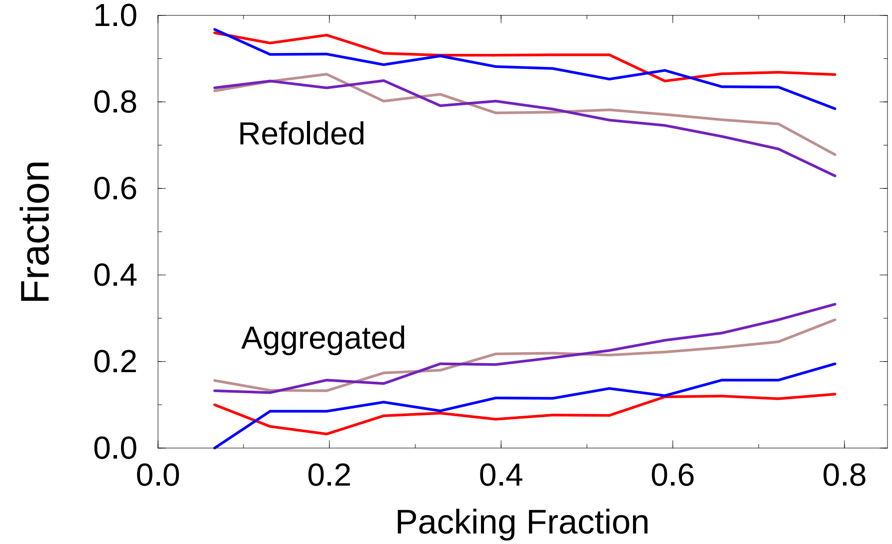
- Refolding yields depend on addition rate

- 1 chain added per pulse:



- Refolding yields are very high

- 5 chains added per pulse:



Conclusions

- Maximum that appears in quenching disappears after 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

Acknowledgements

- National Institutes of Health
- National Science Foundation