# Simulation of Lysozyme Refolding using Simulated Moving Bed Chromatography

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Protein refolding is still one of the baffles for large-scale production of valuable proteins expressed as inclusion bodies in *Escherichia Coli.* Usually biologically active proteins cannot be obtained with high yield at a high concentration and high productivity after refolding. A new continuous refolding method using four zone SMB(simulated moving bed) process based on size exclusion mechanism was developed to overcome these difficulties.

Batch size exclusion chromatography was used to estimate thermodynamic and kinetic parameters for SMB operation. Sephacryl S-100 HR (bead size 47 ㎛) was used as size exclusion medium and packed in glass column (1.6 × 40 ㎝).

With reference to the batch experiment results and operating conditions for SMB from linear triangle theory, the simulation was performed by Aspen chromatography™. Feed was 10 ㎎/㎖ denatured and reduced lysozyme and desorbent was refolding buffer (0.1*M* Tris-HCl, pH 8.2, 1m*M*, EDTA, 3m*M* reduced glutathione and 0.3m*M* oxidized glutathione, 2*M* urea). Feed flow rate was 0.05 ㎖/min and switching time was 85.5 min. The simulation yielded the refolded lysozyme purity to be 99.8 % and the concentration to be 8.06 ㎎/㎖. Protein refolding using size exclusion SMB enables to obtain refolded protein continuously with high productivity, low consumption of refolding buffer, and high efficiency of size exclusion medium.

## References

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