A new method for cell-free production of recombinant proteins containing multiple disulfide bonds

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Due to its highly reducing environment, in general, cytoplasm of *Escherichia* coli does not allow the formation of disulfide bonds. Therefore, expression of recombinant proteins containing disulfide bonds has been a challenging task for biochemical engineers.
In this study, we could produce active recombinant plasminogen activator in a modified cell-free protein synthesis system. A simple treatment of the S30 extract with oxidized glutathione abolished reductase activity while maintaining the translational activity. Addition of DsbC (an *E.coli* protein disulfide bond isomerase), GroEL/ES (an *E.coli* chaperonin) and the optimal concentration of glutathione buffer increased solubility and activity of the product. Approximately 15 μg/mL of active rPA was produced after a 3 hour incubation of the modified cell-free synthesis reaction.