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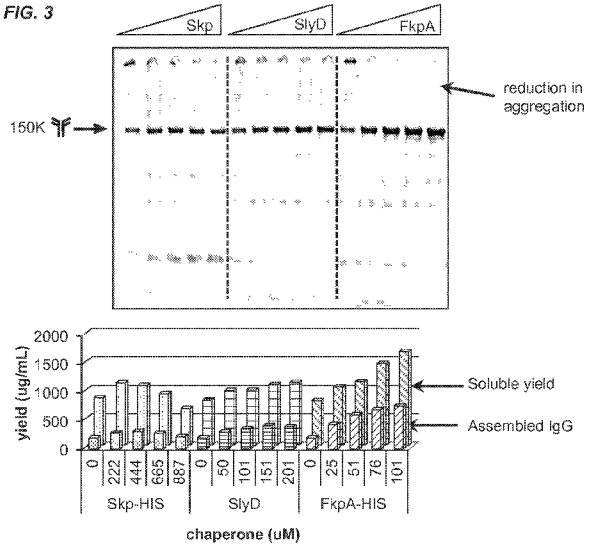
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[Continued on next page]

(54) Title: EXPRESSION OF BIOLOGICALLY ACTIVE PROTEINS IN A BACTERIAL CELL-FREE SYNTHESIS SYSTEM USING CELL EXTRACTS WITH ELEVATED LEVELS OF EXOGENOUS CHAPERONES

FIG. 3



(57) Abstract: The present disclosure describes methods and systems for improving the expression of a properly folded, biologically active protein of interest in a cell free synthesis system. The methods and systems use a bacterial cell free extract having an active oxidative phosphorylation system, and include an exogenous protein chaperone. The exogenous protein chaperone can be expressed by the bacteria used to prepare the cell free extract. The exogenous protein chaperone can be a protein disulfide isomerase and/or a peptidyl-prolyl cis-trans isomerase. The inventors discovered that the combination of a protein disulfide isomerase and a peptidyl-prolyl cis-trans isomerase produces a synergistic increase in the amount of properly folded, biologically active protein of interest.

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