（様式 10）

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（創薬等ライフサイエンス研究支援基盤事業）補助事業成果報告書

I. 基本情報

事　業　名：創薬等ライフサイエンス研究支援基盤事業（創薬等支援技術基盤プラットフォーム事業）
Platform Project for Supporting Drug Discovery and Life Science Research
(Platform for Drug discovery, Informatics, and Structural life science)

補助事業課題名：（日本語）創薬等支援のためのタンパク質立体構造解析に資する高品質タンパク質調製法および結晶生産技術による支援と高度化
（英　語）Preparation of high-quality protein and crystallization techniques for structural analysis of protein for drug discovery

補助事業担当者　（日本語）東京大学大学院新領域創成科学研究科　教授　上田 卓也
所属 役職 氏名：（英　語）Takuya Ueda, Professor,
Graduate School of Frontier Sciences, The University of Tokyo

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II. 成果の概要（総括研究報告）

＜上田グループ＞
これまで PURE system によるタンパク質生産支援のために、コドン最適化や翻訳開始を促進する配列挿入など発現コンストラクトの最適化に取り組んできた。また、EF-P、DnaK、DsbC などの因子が可溶性タンパク質の生産に効果的であることが明らかとなった。最新バージョンの PURE system を使用することによりサブミリグラムオーダーの合成量を達成し、従来の手法では困難であった膜タンパク質など高難易度タンパク質の大量発現が可能になった。GPCR 等のヒト膜タンパク質を人工脂質膜に挿入させた可溶性タンパク質として発現させることに成功し、依頼者にタンパク質試料を提供してきた。例えば、ペプチドホルモンをリガンドとする GPCR の PURE system による発現、可溶化、精製支援を実施した。東工大の田口研究室との共同研究では大腸菌の膜タンパク質の PURE system とリポソームの系での網羅的な発現を行い、現在のところすべてタンパク質で膜に挿入された形で合成されていることが示された。高度化研究では、酵母由来の因子のみで再構成し

た酵母 PURE system の開発を進めた。大腸菌だけでなく、酵母の無細胞翻訳システムを活用することで広範囲のヒト・病原菌由来遺伝子の発現が可能になることが期待できる。また、リボソームディスプレイ法により GPCR のアゴニスト、アンタゴニストの開発につながるペプチド性バインダー因子の取得に取り組んできた。

<田之倉グループ>

本補助事業では、38 サンプルのタンパク質発現支援、16 サンプルのタンパク質精製支援、14 サンプルのタンパク質結晶化支援、14 サンプルのタンパク質性状評価支援を行った。例としては、山越主任研究官（国立感染研究所）らのグループに対し、サイトカインとその疾患関連変異体の発現・高圧巻き戻し技術によるタンパク質の可溶化・精製・結晶化支援を行った。その結果、サイトカインの構造決定に成功し、進化的な機能変換に関わる構造基盤が明らかになった。また、松岡茂特任准教授（大阪大学大学院理学研究科）らのグループに対し、CP/MAS 法を用いた固体 NMR 測定に供するための大型磁場配向結晶を提供した。また別の支援課題においては、分泌型ホスホリパーゼの発現・精製支援を行った。その結果、活性測定試験により、分泌型ホスホリパーゼの一種が新規の活性をもつことが見出された。

磁気力場中での結晶化スクリーニングのハイスループット化に関しては、磁気力場中で多数の結晶化条件を検討可能なサンプル封入容器を開発することに成功し、１度の結晶化実験で検討可能な条件数を従来の４倍に高めることに成功した。また、大型結晶の生成を目的とした実験に対応するため、結晶生成ウェルの容量を大きくした容器も利用できるようにした。その他、磁気力場発生装置を活用した結晶化技術開発として、蒸気拡散法だけでなく、バッチ法・キャピラリー法といった結晶化法にも対応し、高磁場・磁気力ゼロの実験環境も利用できるようにした。以上のように、高品質結晶を得るための結晶化法として磁気力場中結晶化装置を利用した支援体制を拡充した。

高圧巻き戻し技術の高効率化では、全自動で圧力制御が可能な高圧巻き戻しシステムのデザイン及び導入を行い、大容量の実験環境を構築できた。また高圧環境を高効率に運用するための、ハイスループットスクリーニング用デバイスの開発に成功した。本事業で構築した高圧巻き戻し技術を運用することによって、封入体を形成する高難度タンパク質の調製を支援できる技術基盤が整った。

＜中村グループ＞

課題内の東大・上田教授（新領域創成科学研究科）、東工大・田口教授（科学技術創成研究院）、慶大・光武講師（理工学部）らのグループと連携して、大腸菌タンパク質の solubility の網羅的データ eSOL を解析し、配列・構造情報の凝集性への寄与を明らかにした。またこれらの知見をもとに凝集性等予測システム pSOLUB を開発・公開した。pSOLUB は点変異に対する solubility 変化の予測も従来法と同等かそれ以上の性能を示した。この pSOLUB を約 14 万個のヒト ORF に適用し、情報拠点・お茶女大・由良教授（理学部）と連携して、結果を情報拠点の VaProS システムに提供した。また、上田教授、田口教授、京大・秋吉教授（工学研究科）らのグループとの連携で、liposome 存在下における膜タンパク質凝集性予測システム pSOLUB_mem を開発した。さらに、課題内の東大・田之倉教授（農学生命科学研究科）のグループとの連携により、高圧巻き戻しデータの予測可能性を明らかにし、データ蓄積および検索を可能としたシステム・インターフェースを開発した。

<Ueda group>

To improve protein production by PURE system, we have been working on optimization of DNA constructs by codon optimization and inserting AT-rich sequence at 5' region which promotes translation initiation. Also, protein factors such as EF-P, DnaK and DsbC turned out to be effective to enhance productivity. With this improved PURE system, we achieved sub-milligram protein production, and it is now possible to overexpress proteins which has been considered difficult to express in vitro (e.g. membrane proteins). Since membrane proteins are prone to aggregate in vitro, expression was performed in the presence of a form of lipid which is called nanodisc. As an example, we could express and purify a series of GPCR, ligand of which is a peptide, in PURE system. In collaboration with Professor Taguchi's laboratory (Tokyo Institute of Technology), we performed extensive analysis of PURE synthesis and membrane insertion of E.coli membrane proteins. All membrane proteins tested were found to be integrated into liposome. We also developed another PURE system which is reconstituted with yeast translation factors. This new yeast PURE system together with E.coli PURE system would make it possible to express wider range of proteins including ones from human and pathogenic bacteria. Furthermore, we have been working on developing ribosome display to obtain peptide binder which binds to GPCR. Such a binder could lead to discovery of agonist or antagonist.

<Tanokura group>

In the subsidy project, we supported 38 protein expression, 16 protein purification, 14 protein crystallization and 14 protein evaluation. For example, we succeeded in preparing a cytokine and its mutants related to diseases such as rheumatoid arthritis by using high pressure refolding system. The purified proteins were provided for functional studies by Dr. Yamagoe (Infectious Disease Surveillance Center). In addition, we supported structure determination of the cytokine and then revealed a mechanistic basis of functional evolution that could be related to the physiological function of mammalian protein with an M23 metalloendopeptidase fold. Using the magnetic force field environment, we supported Dr. Matsuoka of Osaka University to obtain large protein crystals showing magnetic orientation for solid-state CP/MAS NMR spectroscopy. As another example, we supported the preparation of secretory phospholipase A2 (sPLA2) by using high pressure refolding system, which contributed to find out the novel function of sPLAs.

In the subsidy project, we developed the high throughput protein crystallization system in magnetic force field and the effective high pressure refolding system. In the development of the protein crystallization in magnetic force field, we improved a crystallization device (container) enabling high-throughput crystallization (4-times as much as a conventional one) or large-scale crystallization. We can utilize not only the vapor-diffusion method but also the batch method and counter-diffusion method in the magnetic force field. In the development of the high pressure refolding, we designed and developed a new high pressure refolding system that enables automated pressure regulation. We also developed a new high throughput devise that can set up multiple samples easily. We have supported clients using this system and solubilized 13 protein samples that were expressed as inclusion bodies.

<Nakamura group>

We analyzed the relationship between protein solubility and sequence/structure information based on eSOL database comprehensively, and developed the protein solubility prediction system called "pSOLUB" based on these findings. We showed that pSOLUB can predict the deviation of solubility on mutation comparable to or better than the previously reported system. We provided predicted solubility data of 140 thousand human ORFs to VaProS system. Also we developed the solubility of membrane proteins with liposome. In addition to that, we showed the possibility to predict the high-pressure refolding data and developed interface for the data accumulation and retrieval.

III. 成果の外部への発表

（１） 学会誌・雑誌等における論文一覧（国内誌 0 件、国際誌 70 件）

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（４）特許出願