Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Engineering of protein assemblies within cells

Tien K Nguyen and Takafumi Ueno

Recent achievements in development of protein assembles within cells have extended biosupramolecular composites into a new era with versatile applications in the fields of biomaterial and biotechnology. Using methods with biological and physicochemical routes has made this era of research more interesting and challenging. Further advances in protein engineering have facilitated efficient fabrication of supramolecular complexes within living cells. Here, we provide a review of recent efforts to engineer protein assemblies within cells and describe the promising properties of these assemblies.

Address
Department of Life Science and Technology, School of Life Science and Technology, Tokyo Institute of Technology, Yokohama 226-8501, Japan

Corresponding author: Ueno, Takafumi (tueno@bio.titech.ac.jp)

Current Opinion in Structural Biology 2018, **51**:1–8

This review comes from a themed issue on **Engineering and design**

Edited by **Giovanna Ghirlanda** and **Ivan Korendovych**

https://doi.org/10.1016/j.sbi.2017.12.005

Introduction

Proteins play central roles in biological systems by contributing to cellular processes such as signal transduction, cell cycle regulation and chemical transportation [1]. Within cells, proteins may be expressed at high concentrations, under conditions which can promote self-assembly into precise subcellular organizations as metabolic organelles [2,3], or container of molecules [4,5]. Such protein assemblies have the potential for use as rationally designed nano-scale materials [6]. Therefore, engineering of proteins within cells is a research area which supports fundamental investigations of protein assemblies and development of innovative materials within a cellular environment [7••].

Recent achievements have highlighted a growing interest in conversion of natural assemblies to artificial assemblies under cellular conditions. The local protein concentration promotes the evolution of intermolecular protein–protein interactions into defined organizations, with evidences of discrete (icosahedral cages, spherical cages, oligomeric states) [7••,8••,9••] and infinite structures (crystal, lattice-like, filamentous) [10••,11•]. These insights have been facilitated by studies involving biological and physicochemical synthetic routes [9••,12•,13•,14]. During protein overexpression, the proteins are folded, increased in concentration and induced to self-assemble via intermolecular contacts mediated by coordination of metals and non-covalent interactions [9••,12•]. Engineering of protein assemblies has enabled rapid development of such useful materials for applications in bioscience and biotechnology. Examples include *in vivo* dynamic protein crystallization [15•], nanoplatform for molecular delivery [8••], enzymatic activity [9••,13•], and molecular encapsulation [10••,16,17•]. Most recently, it has been reported mutation of surface of oligomer triggered evolution of various proteins into supramolecular assembly within cells [18]. Here, we review recent achievements in engineering of protein assemblies within the intracellular environment (Figure 1).

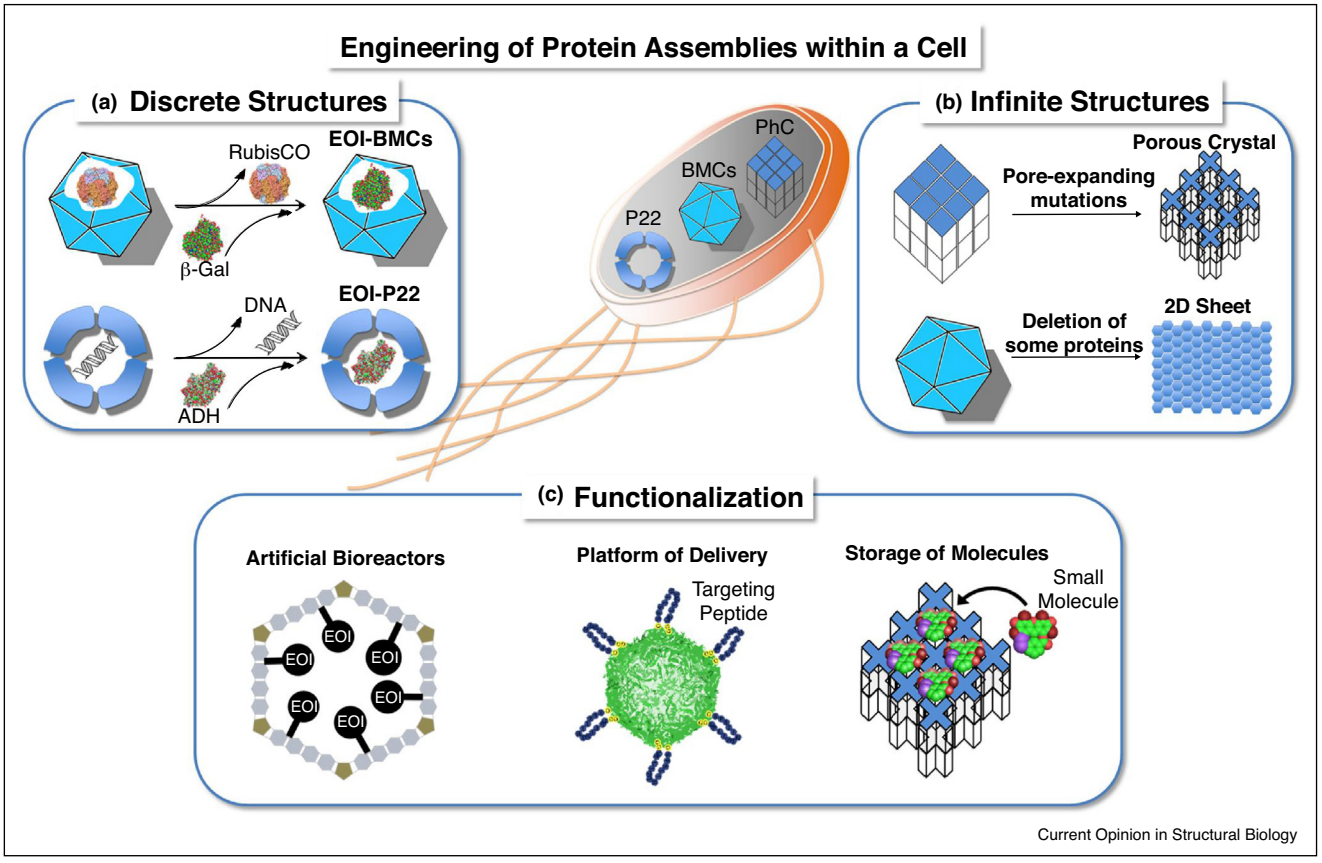
Engineering of microcompartment shell proteins under intracellular overexpression conditions

Bacterial cells can contain microcompartments (BMCs) as inclusion bodies confirmed by electron microscopy (TEM) [2,18]. BMCs are formed of various shell proteins organized into icosahedral structures with encapsulating enzymes [2]. Toward a goal of compartmentalization, engineering of BMCs shell proteins within cells can lead to a synthetic strategy for constructing artificial scaffolds for bio-related applications [18,19].

Warren *et al.* conducted pioneering investigations in construction of an empty icosahedral structure of BMCs in *Escherichia coli* by encoding of coordinated genes from BMCs of PduA/J/K/N (Figure 2a) [7••]. Expression of one gene or selected genes from BMCs prevented a generation of BMCs, but resulted a revolution into intensive assemblies with infinite structures (Figure 2b) [7••,20]. PduA can contact itself or other shell-proteins in different routes to produce rolled-up, filamentous, and lattice structures, respectively with PduA/B/J, PduA/B and PduA [7••]. Amino acid replacement of PduA leads to transformation into a rolled-up structure with V51A and a layer structure with K26A or R79A [11•]. These findings strongly demonstrate the potential for engineering of a single BMC shell-protein to develop cellular assemblies [20–22].

Compartmentalization provides a powerful driving force for encapsulating materials (Figure 2c) [7••,16,23,24].

Figure 1



Engineering of protein assemblies within cells. Using natural protein assemblies discovered in cells, artificial architectures are constructed by protein engineering, via either biological or physicochemical routes. They are classified as having **(a)** discrete structures and **(b)** infinite structures. Natural systems with discrete structures were re-constructed with artificial scaffolds by EOI [16,24]. For infinite structures, engineering of proteins could change their properties (e.g. porous crystal) [10••], and their morphologies (e.g. from icosahedral to 2D sheet) [7••]. **(c)** Protein assemblies have been functionalized as artificial biomaterials, such as bioreactors [24], platform of delivery [8••], and storage of molecules [10••]. Abbreviations used in this figure: BMCs = bacteria microcompartments, EOI = enzyme of interest, P22 = bacteriophage P22, RubisCO = ribulose-1,5-bisphosphate carboxylase/oxygenase, ADH = alcohol dehydrogenase, PhC = polyhedra crystal.

A co-expression system with fused green fluorescent proteins (GFPs) was imaged in *E. coli*, demonstrating that these enzymes become localized inside the BMCs [7••,23]. Further engineering of bacterial compartments by fusion of exogenous enzymes generated nano-reactors [16,24]. These enzymes were held within BMCs, which could enhance their stability and promote the metabolism [16,24]. Characterization of PduA include of ~20 nm hollow-tubes which are promising materials for molecular encapsulation [11•].

Overexpression of high concentrations of proteins provides subcellular conditions which induce development of protein assemblies in living cells. Through spatial engineering of proteins, cells may be organized into discrete or infinite structures. This is one of the principles used in development of biomaterials for molecular encapsulation and regulation of cellular processes.

In vivo-encapsulation of exogenous materials within a protein-cage

Spherical protein cages composed of homo or hetero subunits form discrete architectures [25,26]. These structures are promising candidates as the delivery platforms for biomedical applications [25]. To date, many proteins have been demonstrated to assemble into cages within cellular environments. Examples of such self-assembling proteins include bacteriophage P22, encapsulin, and lumazine synthase (Figure 3a) [4,27,28]. Engineering of protein cages within cells is expected to provide a new approach for development of versatile templates in biotechnology (Figure 3b,c) [8••,29••,30,31•,32].

Kang *et al.* engineered an encapsulin cage from *Thermotoga maritime* as a cargo delivery system [8••]. When encapsulin was genetically fused with the targeted fragment of SP94-peptide, the recombinant protein was