Cloning & Expression System for Repetitive Units & relevant Products

All-in-one vector toolbox - straightforward cloning, multimerization, combination & expression of repetitive protein polymer units & their elastin-like products

Technology

Protein-based biomaterials and particularly **peptide based polymers** are of great interest for the biomedical field and open up a wide class of potential new applications, e.g. use as prosthesis, scaffolds in tissue engineering, drug release or polymers in non-medical fields.

Here, **1)** a fast and reliable protected **method - a one vector toolbox platform -** for cloning, assembly, modification and expression of protein-encoding polymers - up to very large constructs, as well as **2) our skills & biomaterial systems** for precision protein engineering & production and **3)** resulting relevant **polymeric products** (i.e. elastin-like proteins, amphiphilic a/o of resilintype) are introduced. *Continued on p.2*

For all of the above (1-3) we are in search for R&D sponsoring & cooperation partners

Innovation/ Key Features of Method

- Easy to use construction kit with functional and structural tectons
- Classical cloning strategy - not PCR based, hence sequence reliability
- No re-cloning - smart *all-in-one* cloning & expression system
- Restriction enzymes or annealing of synthetic ssDNA with self-complementary overhangs generate dsDNA template monomers
- Standardized overhangs allow free combination of different monomers
- Overhangs in frame encode for glycine - appearing in almost all structural proteins that are potential targets for polymerization

Main Applications of Method

Access to the production of biopharmaceuticals, recombinant peptides or polymeric protein-based biomaterials for plastic surgery, tissue replacement, chronic wounds, cartilage replacement, drug formulation and release strategies or ECM mimicry for cell culture and stem cell niches.

Developmental Status

- High **method** reproducibility with all monomers tested so far
  - up to 8.5 kb (300 bp units) cloned, multimerized & expressed
  - vectors (with or without His-Tag) validated for E. coli and insect cell expression (inducible or constitutive)
  - huge library of available functional and structural tectons (architectural building blocks) for complex protein polymers
- Several **skills**, **biomaterial systems** & validated **products** (see p. 2)

Literature

- Huber et al., Nature Materials 2015, 14, 125–132
- Huber et al., Biomaterials 2014, 35, 8767-8779
- Schiller, in Synthetic Biology, ed. Giese, B., von Gleich, A., Pade, C., Wigger, H. (Springer, 2014, ISBN 978-3-319-02782-1)

Responsible Scientist

Dr. Matthias Huber & Dr. Stefan Schiller

Freiburg Institute for Advanced Studies (FRIAS) & Center for Biological Systems Analysis (Zentrum für Biosystemanalyse; ZBSA)

Albert-Ludwigs-University, Freiburg

Branch

BioTech - Peptide Based Polymers

Patent Status

Method:

EP 2668962 (PRD) May 29th 2012, granted and validated

Products:

PCT filing date May 28th 2013
PCT (WO2013178627) nat./ reg. in

- EP (2854863), granted and validated

- US (US2015126452), pending

Reference Number
ZEE20111102

Status: Oct. 2018

CTF – The R&D Company of the Freiburg University and the Freiburg University Medical Center

Contact
Dr. Wolfgang Jost
Campus Technologies Freiburg GmbH
Stefan-Meier-Str. 8 | D-79104 Freiburg
Email: Wolfgang.Jost@campus-technologies.de
Tel: +49 (0)761 203-97754
Fax:+49 (0)761 203-5021

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Technology

Continued

1) Method: Our novel „one vector toolbox platform“ enables the straightforward construction, production and functional testing of molecular tecton libraries. Homo- or hetero-polymeric DNA-templates (DNA-tectons) for structural and functional protein polymer domains (protein-tectons) with different properties or block-copolymers with tailored behavior are accessible. All of the resulting DNA-tectons are compatible to each other by means of having standardized complementary overhangs and therefore could be combined and spatially arranged without any limitation. Thus, functional sequences (e.g. enzymatic functions, protease recognition sequences, etc.) successfully could be integrated into various structural protein polymers. On top, the insertion of spatially defined functional groups or amino acids (e.g. disulfide bonding by amino acid cysteine, unnatural amino acids) allows for the defined covalent bonding of individual protein polymer domains to generate branched protein (polymer) complexes with defined structural and functional capabilities or to functionalize proteins with chemically synthesized adducts or for surface interactions.

2) Skills & Biomaterial Systems for precision protein engineering & production:
- designable amphiphilic peptides & proteins (adjustable in length, chemical composition e.g. cationic, anionic and neutral amphiphiles/ detergent properties)
- protein-based elastic materials & hydrogels with adjustable E-modulus and resilience (for technical application) & selective epitopes introduction (for regenerative medicine/ tissue replacement or engineering)
- design & synthesis of highly repetitive proteins e.g. structural proteins, proteins of the extracellular matrix or oligomeric repeats of small peptides
- design and production of peptides lager than 10 amino acids (drugs, surface active and protecting)
- production of DNA/ aptamers, above 100 pb up to several kbp’s e.g. for scalable aptamer production, DNA Origami or other DNA-based applications

All protected by our patented „one vector toolbox platform“ allowing to selectively access highly repetitive symmetric or asymmetric DNA/protein sequences which can be combined symmetrically and asymmetrically up to large sizes.

3) Products: Based on the above skill-platforms (2) following products & applications are in our focus:
I) a) Encapsulation and protection of small molecules, proteins, enzymes and whole cells via our ultra-stable but dynamic protein vesicles; **b)** drug formulations; **c)** protein amphiphiles as bio-based & biodegradable detergents or as surface modification agents to facilitate adhesive properties in gluing applications
II) Protein-based elastomers with adjustable elastic modulus & low energy dissipation for technical applications & due to high biocompatibility beneficial applications as hydrogels in regenerative medicine
III) Biopharmaceuticals and novel oligomeric drugs
IV) Design and production of DNA/ aptamers, above 100 bp & from 10 mg to low gram scales

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Freiburg University and the Freiburg
University Medical Center

Contact
Dr. Wolfgang Jost
Campus Technologies Freiburg GmbH
Stefan-Meier-Str. 8 | D-79104 Freiburg
Email: Wolfgang.Jost@campus-technologies.de
Tel: +49 (0)761 203-97754
Fax:+49 (0)761 203-5021