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Research Article

LOCKR - A *De Novo* Biological Switch for Cellular Feedback Control: A Meta-Analysis Research

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Abstract

Background: Designed proteins hold great promise as building blocks for synthetic circuits, one such designer protein-degron LOCKR, which is based on 'latching orthogonal cage-key proteins' (LOCKR) technology-is a biological switch that degrades a protein of interest *in vivo* via a genetically encoded peptide. The designed nature of degron LOCKR proteins enables modifications to tune feedback behavior. This work demonstrates the large and untapped potential of *de novo* design of proteins for generating tools that implement complex synthetic functionalities in cells for a myriad of applications.

Materials and Methods: With the Medline database, Cochrane collaboration and Medknow database taken as a source for authenticated scientific research data, 70 articles were selected having undergone randomized control trial. Out of these, 17 articles (studies) were chosen which met the criterion for meta-analysis.

Results and Conclusion: LOCKR can act as a molecular switch. Individual LOCKR proteins can also be connected to form circuits that induce intracellular changes in response to both internal and external stimuli. The ability to engineer feedback control into living cells represents an important milestone in biology. LOCKR's potential applications are endless, including biotechnological and therapeutic applications that can change the vista of cellular signaling.

Keywords: LOCKR; De Novo; Molecular Switch; Feedback Control; Meta-Analysis

Introduction

The functionality of the human body is the result of the interplay of more than twenty thousand different types of proteins. Our understanding and perception of therapeutic measures for maladies has changed over the past few decades, since the advent of molecular interventions and gene therapy as treatment modalities. Endogenous proteins play a major role in the regulation and maintenance of homeostatic mechanisms. Several attempts have been made over the years, to modify endogenous proteins in order to achieve superior outcomes. Although the results have not been satisfactory due to the wide variety of possible conformational changes in endogenous proteins that enable multiple undesirable interactions and the resultant binding to unintended protein domains (off-target binding). To overcome the demerits of endogenous protein modification, *de novo* protein design technology has evolved.

As per the American Chemical Society, Protein design is defined as "the rational design of new protein molecules to design novel activity, behavior, or purpose, and to advance basic understanding of protein function".

Proteins can be designed from square one (*de novo* design) or by making variants of a known protein structure (protein redesign). Protein design aims at predicting amino acid sequences that will fold to a desired three dimensional structure. The native state of a protein is the conformational free energy minimum for the chain or the lowest energy state of thermodynamic stability. Protein design is the reverse process of protein structure prediction, wherein the

3D structure (tertiary structure) of the protein is specified, and a sequence that will fold to the desired structure is identified. Hence, it is also termed inverse folding. Protein design is then an optimization problem: using some scoring criteria, an optimized sequence that will fold to the desired structure is chosen.

"*De novo*" proteins are synthetic, computationally engineered designer proteins, designed by computational protein design technology, to perform specific functions. In our meta-analysis research, we have explored newest breakthrough in *de novo* protein design, LOCKR.

Aim of the Study

To determine the significance of LOCKR in rewiring complex endogenous signaling pathways and evaluate feedback control.

Research question

Can LOCKR act as a tool to engineer feedback control in living cells for biotechnological and therapeutic applications?

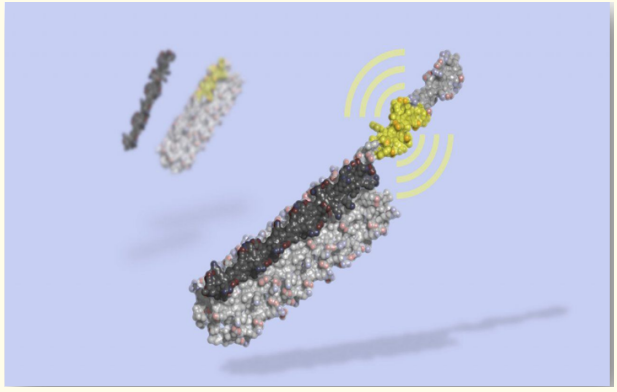


Figure a

Evolution of '*de novo*' protein design

Table 1

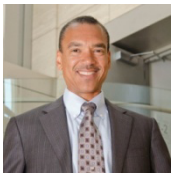
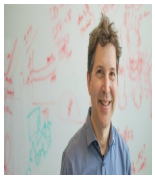
Timeline	Advancement	Contributors
1970s to 1980s	First rationally designed proteins (reduced bovine ribonuclease etc.)	Bernd Gutte, Urry, Richardson., *et al*.
1997	First protein successfully designed completely '*de novo*'	Stephen Mayo **and coworkers** 
1999	Dimers, trimers, and tetramers of unnatural right-handed coils	Peter S. Kim **and coworkers** 
2003	Completely synthetic protein with structure never seen before in nature	David Baker and coworkers 
2008	Computationally designed enzymes	David Baker and coworkers
2010	Neutralizing antibodies isolated from patient serum using a computationally designed protein probe	David Baker and coworkers

Table 1: Timeline depicting the evolution of '*de novo*' protein design.

What is LOCKR?

LOCKR stands for Latching, Orthogonal Cage/Key pRotein.

LOCKR technology consists of three major components:

- Cage
- Designer Switch
- Key/inducer protein.

LOCKR cage

It is a static, five-helix 'cage' or five helix 'barrel', enclosing a bioactive peptide preprogrammed to perform a specific function such as the initiation of transcription, targeted protein degradation, triggered apoptosis etc.

Designer switch

It is a six helix bundle, with a destabilized sixth helix which acts as the 'latch'.

LOCKR key

It is an exogenous, de novo inducer protein which outcompetes the latch to bind to the cage, thus releasing the bioactive peptide to generate a desired output.

Degron LOCKR

Degron LOCKR is based on LOCKR technology.

It consists of two major components:

- Designer degron Switch
- Inducer protein (key).

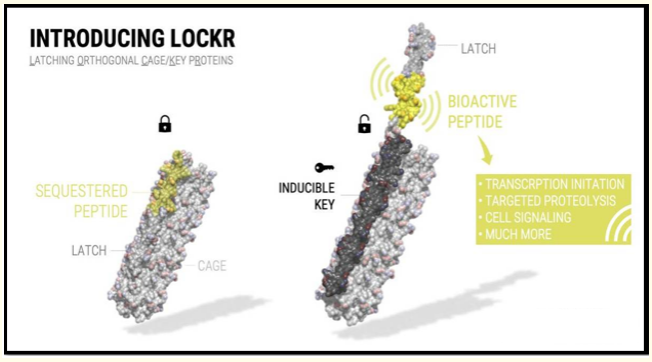


Figure b

Degron: It is a portion of a protein that regulates protein degradation rates.

Examples

Known degrons include short amino acid sequences, structural motifs and exposed amino acids (often Lysine or Arginine) located anywhere in the protein.

Degron mechanisms

They are categorized based on their dependence on Ubiquitin, (a small protein involved in proteasomal protein degradation), as "Ubiquitin-dependent" or "Ubiquitin-independent".

Degron-Switch

It is a six-helix bundle that has the degron embedded in the destabilized sixth helix (the 'latch'), as part of the five helix bundle/ Cage in the 'closed' state of LOCKR protein.

Inducer protein (Key)

The key is an exogenous designer protein that can outcompete the latch for binding with the cage, which exposes the degron and targets the degron Switch for degradation, rendering the LOCKR protein in an 'open' state. The degron LOCKR Key, can be genetically encoded.

The binding of Key to the Cage occurs through a 'PLUG AND PLAY' mechanism as illustrated.

Modular feedback control is achieved by directly fusing the Degron Switch to a protein of interest in a biological network and expressing the key via the transcriptional output of the network.

As shown in the schematic diagram below, the biological network is represented by a series of proteins A through Z, which interact among themselves to generate a given output. Modular feedback control is achieved by fusing the 'designer switch' to the protein of interest B and generating the 'key' via the transcriptional output of the network. The 'key' then binds to the 'cage', releasing the bioactive peptide to generate a desirable output.

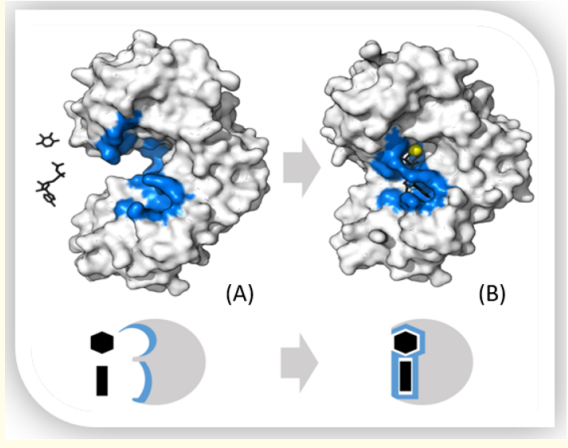


Figure 1: Plug and play' mechanism of binding of LOCKR key to cage.

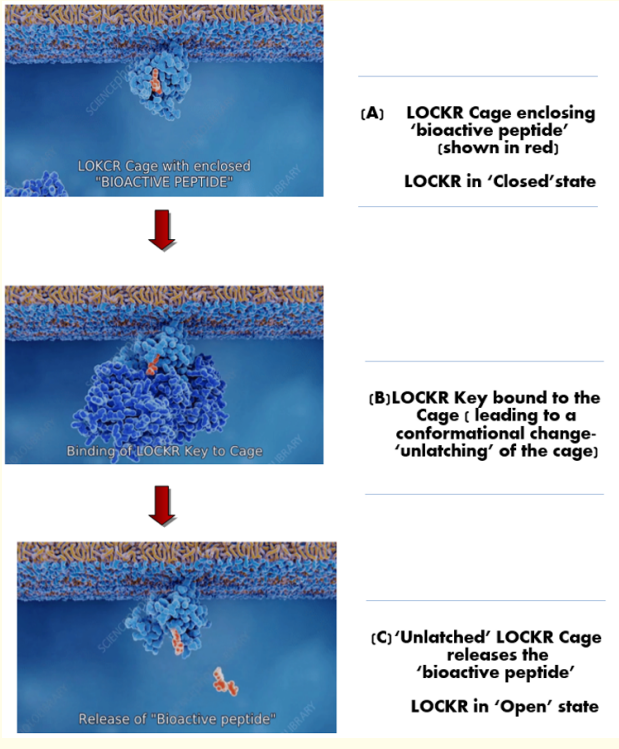


Figure 2: Mechanism of confirmational change of LOCKR protein from 'closed' to 'open' state.

Aims and Objectives

To statistically establish the significance of LOCKR as a tool to engineer feedback control in living cells for biotechnological and therapeutic applications using Meta-Analysis.

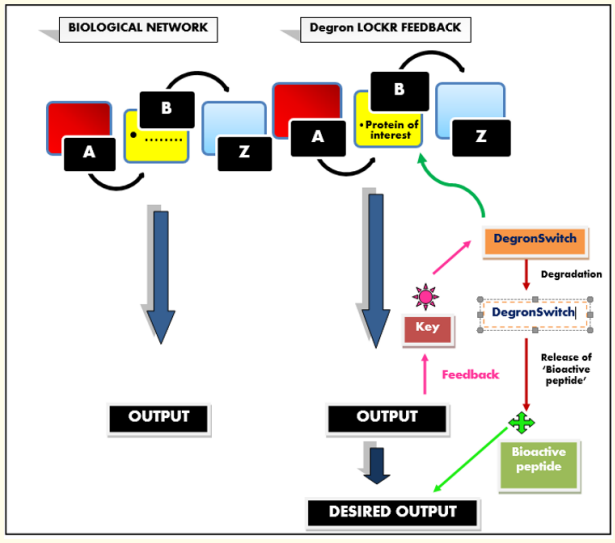


Figure 3: Schematic diagram showing mechanism of modular feedback control achieved via LOCKR technology.

The objectives of the study included:

- To determine the significance of LOCKR in rewiring complex endogenous signaling pathways.
- To evaluate feedback control achieved using LOCKR technology.

Materials and Methods

This review follows international guidelines for performing and reporting systematic reviews and meta-analysis (Moher., *et al*. 2009; Hoaglin., *et al*. 2011; Jansen., *et al*. 2011). We aimed at answering the following question: Can LOCKR act as a tool to engineer feedback control in living cells for biotechnological and therapeutic applications?

Selection (inclusion and exclusion) criteria

With the Medline database and Cochrane collaboration taken as source for authenticated scientific research data, 70 case control articles were selected having undergone a randomized control trial. Out of these, the articles were screened and finally 23 articles (Table 1) were selected which met the criterion for Meta-Analysis. Articles which were not published in English and where the full text could not be obtained were excluded. Case reports, reviews, non RCTs and non case control articles were also not included in the study. All the articles were checked for validity, eligibility and design of the study.

Results

Meta-analysis is a technique of combining the results of many studies in a rigorous and systemic manner to allow us to better visualize the overall implications of the studies being undertaken.

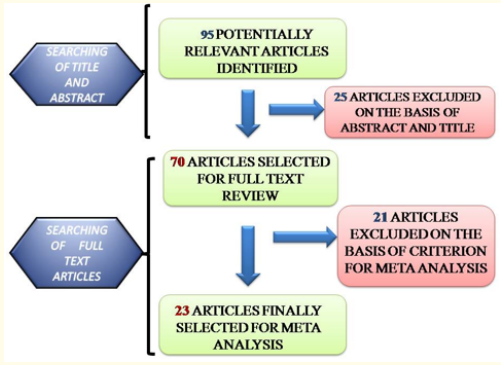


Figure 4: Inclusion and exclusion of articles for the meta analysis.

A Meta-Analysis was carried out with selected studies within a period of 5 years between 2014 to 2019. The Odds ratios and 95% confidence intervals (CI) were calculated. The final probability value obtained after analysis of all the selected 23 studies was less than 0.001 indicating that our study has been statistically significant.

Discussion

Most diseases known to mankind, manifest clinically as a result of pathological proteins produced due to the alteration in physiological mechanisms that maintain homeostasis in the human body. The examples given below illustrate the said statement:

- Misfolding of Chaperone proteins results in dermal and oral manifestations seen in Lichen Planus.
- Misfolded Spectron proteins lead to manifestations of Sickle cell disease.
- Oncoproteins generated due to mutations in tumour suppressor genes and activation of proto-oncogenes lead to varied neoplastic changes, manifesting as cancer.

LOCKR technology can revolutionize therapeutics, as it enables the destruction of such pathological proteins by introducing specific bioactive peptides encoded for targeted degradation of pathological proteins.

Applications of LOCKR technology

The wide panorama of applications that LOCKR technology has in the fields of biomedicine, bioengineering and dentistry is fascinating.

- **Biomedicine**: LOCKR technology can open new doors in long standing medical mysteries such as Oncotherapy and management of autoimmune and neurodegenerative disorders. Newer treatment modalities like 'Smart cell therapies' (E.g. Chimeric Antigen Receptor (CAR) T cell therapies for leukemia) can be enhanced using LOCKR

proteins. Complicated medical conditions like traumatic brain injury can also be effectively managed using LOCKR technology, by introducing 'protein kinks' that act as 'check points' in the inflammatory cascade.

- **Bioengineering:** LOCKR technology permits the control of cellular circuitry via modification of gene expression, redirection of cellular traffic; enabling, disabling or facilitating the transport of molecules intracellularly etc.
- **Dentistry:** Oral medicine will view a paradigm shift in existing treatment modalities as LOCKR technology can play a key role in Oral Oncotherapy and management of Potentially premalignant oral epithelial lesions (PPOELs), autoimmune diseases such as Sjogren syndrome, Oral inflammatory disorders and Oral Implantology by enhancing osteogenesis and osseointegration, thus simplifying to a great level the procedures involved in bone augmentation and implant placement.

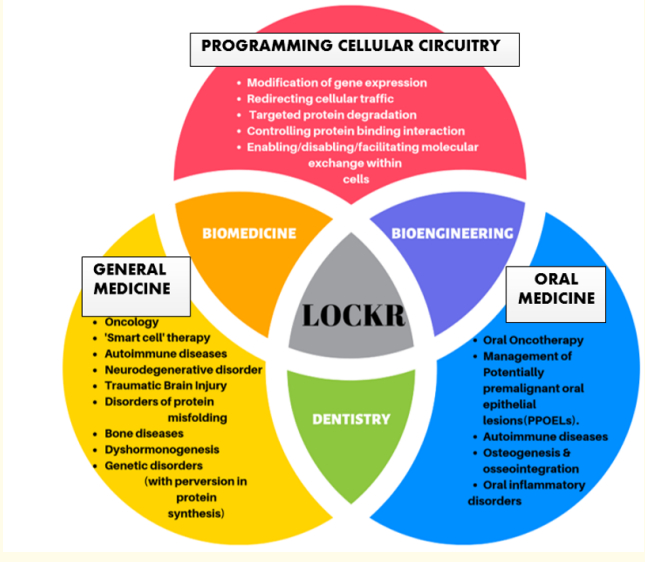


Figure 5: Applications of LOCKR technology.

Conclusion

LOCKR can act as a molecular switch. Individual LOCKR proteins can also be connected to form circuits that induce intracellular changes in response to both internal and external stimuli. Degron-LOCKR technology can be used in *in-vivo* modification of mammalian cells, which opens the door to a wide range of applications in the design of live cell therapeutics [1-16].

The feedback circuits based on degron LOCKR, open avenues for synthetic biology based on designer proteins. A toolkit of *de novo*-

S. No	Researches conducted by	Standard deviation
1.	Seldin., *et al.*	1.38
2.	Steer., *et al.*	1.94
3.	Wesoly., *et al.*	1.37
4.	Zhernakova., *et al.*	1.93
5.	Harrison., *et al.*	1.48
6.	Morgan., *et al.*	1.59
7.	Viken., *et al.*	1.63
8.	Wesoly., *et al.*	1.59
9.	Sahin., *et al.*	1.39
10.	Hinks., *et al.*	2.04
11.	Majorczyk., *et al.*	1.96
12.	Eike., *et al.*	1.79
13.	Farago., *et al.*	2.27
14.	Chabchoub., *et al.*	0.91
15.	Sfar., *et al.*	16.17
16.	Majorczyk., *et al.*	3.85
17.	Plenge., *et al.*	1.32
18.	Orozko., *et al.*	1.50
19.	Pierer., *et al.*	2.57
20.	Johansson., *et al.*	2.64
21.	Kokkonen., *et al.*	1.79
22.	Lie., *et al.*	1.88
23.	Starck., *et al.*	1.68

Table 2: List of articles selected for the study based on meta-analysis criteria.

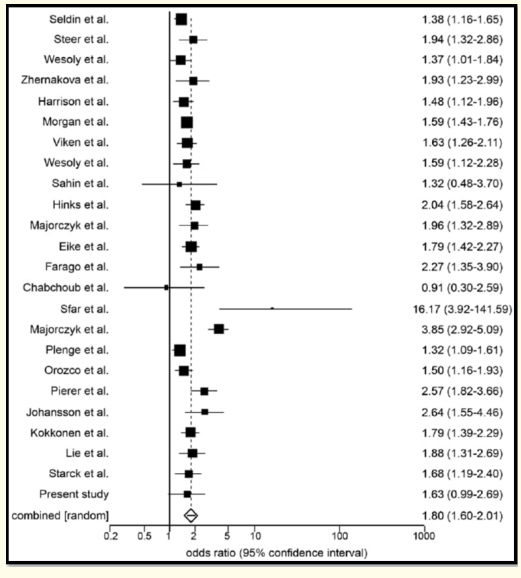


Figure 6: Meta-analysis graph (forest plot) showing the articles selected for the study based on meta-analysis criterion.

designed proteins could catalyze future applications of engineered cells in the same way that modular electronic parts have enabled the expansion of the semiconductor industry.

The ability to engineer feedback control into living cells represents an important milestone in biology. LOCKR's potential applications are endless, including biotechnological and therapeutic applications that can change the vista of cellular signaling.

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