Press Release
Engineered bugs for the discovery of new drugs

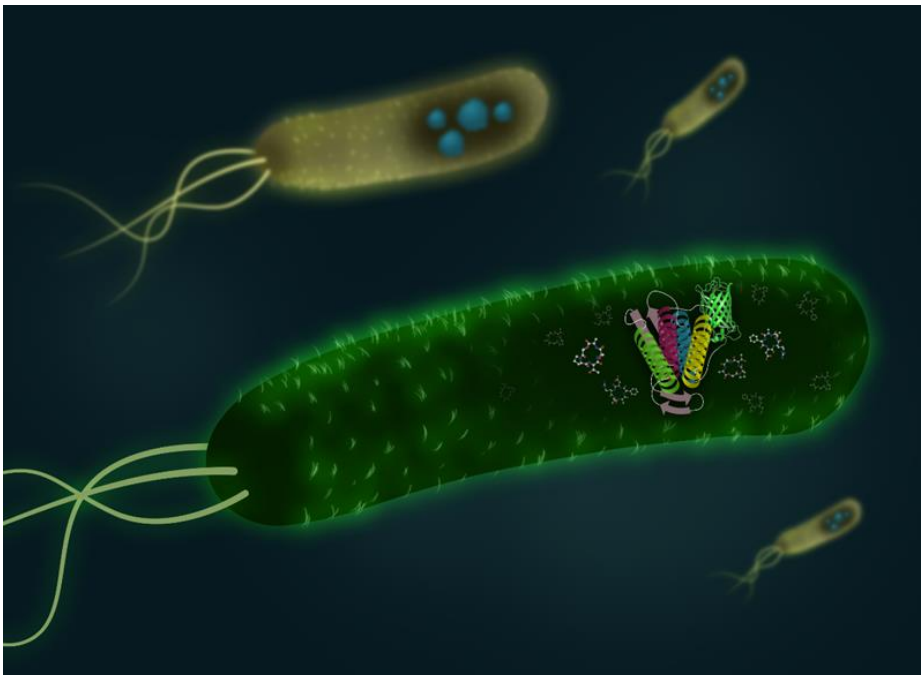


Illustration by John Chaniotis

Athens, 24.10.2017

A variety of human diseases with different pathologies, such as Alzheimer's disease (AD), Parkinson's disease (PD), type II diabetes, cystic fibrosis and others, share a common molecular characteristic: the misfolding of specific proteins. These conditions are collectively termed "protein misfolding diseases" (PMDs). Currently, there are more than 50 human disorders, which are classified as PMDs, and the vast majority of them remain incurable. PMDs include very serious conditions with a severe impact on the well-being of modern societies, and anti-PMD therapeutics are in enormous demand. A very promising approach for the discovery of potential therapeutics against PMDs is the identification of small molecules that bind problematically folded proteins and convert them back to their normal or benign conformations. Researchers of the Institute of Biology, Medicinal Chemistry & Biotechnology (IBMCB) at the National Hellenic Research Foundation (NHRF), led by Dr. Georgios Skretas, have now generated genetically engineered bacteria that enable facile and rapid discovery of chemical rescuers of PMD-associated protein misfolding. In this new system, large combinatorial libraries of tens or hundreds of millions of macrocyclic peptides are biosynthesized in *Escherichia coli* cells and simultaneously screened for their ability to rescue pathogenic protein misfolding and

aggregation using an ultrahigh-throughput assay (Figure 1). The development of the technology and its application to the discovery of potential therapeutics against Alzheimer's disease and amyotrophic lateral sclerosis are reported in the October 2017 issue of ***Nature Biomedical Engineering***.

In order to come up with new ways to discover novel drugs against PMDs, the group of Dr. Georgios Skretas chose to take advantage of the tremendous biosynthetic capabilities of simple microbial cells, such as *E. coli*. To develop a technology where the lead discovery process can take place as rapidly, easily and efficiently as possible, they applied a Synthetic Biology approach to generate engineered bacteria with the ability to perform two important tasks: (1) biosynthetic production of tens or hundreds of millions of different test compounds exhibiting high levels of chemical and structural diversity, and (2) simultaneous detection of the rare bioactive molecules with the ability to rescue the misfolding of the target disease-associated protein efficiently.

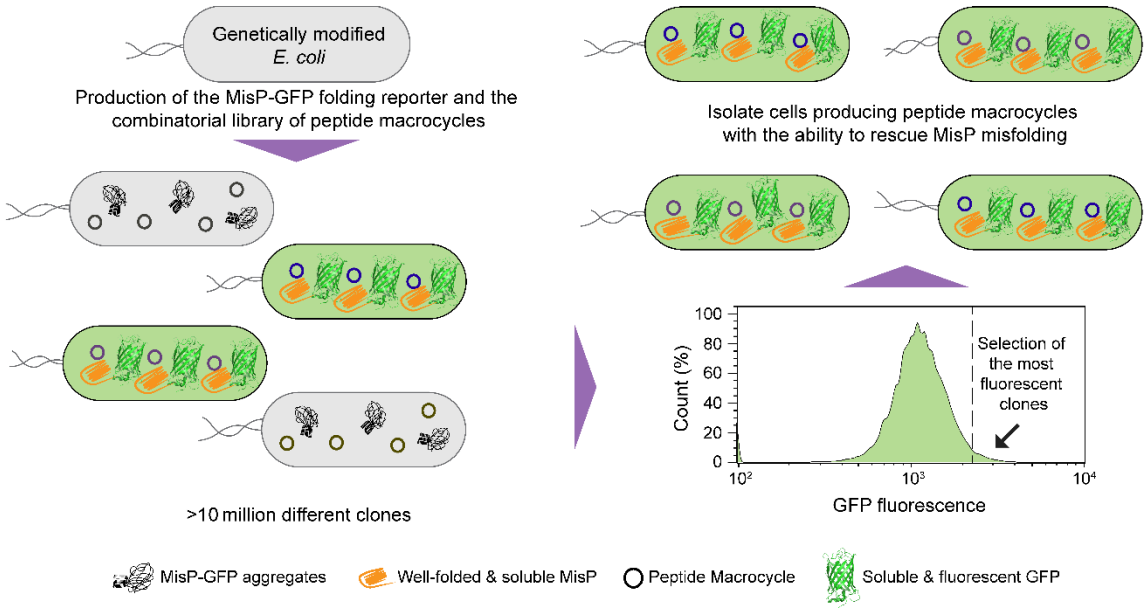


Figure 1: Representation of the bacterial system used for the discovery of macrocyclic rescuers of misfolding and aggregation of misfolding-prone, disease-associated proteins (MisP). Adapted from Matis, I. et al. An integrated bacterial system for the discovery of chemical rescuers of disease-associated protein misfolding. *Nat. Biomed. Eng.* (2017) http://dx.doi.org/10.1038/s41551-017-0144-3.

Using this approach, the NHRF researchers biosynthesized and screened a combinatorial library of >10 million macrocyclic peptides for the identification of inhibitors of neurotoxic aggregation of the β-amyloid peptide (Aβ), which is associated with the etiology of Alzheimer's disease. Through this process, hundreds of different macrocycles were identified that inhibited Aβ aggregation in bacterial cells. Among these, two molecules, termed AβC5-34 και AβC5-116, were synthesized chemically and subjected to a series of

biochemical, biophysical, computational, and biological analyses for evaluation of their properties, in collaboration with other labs from IBMCB-NHRF, the National Centre for Scientific Research "Demokritos", the National & Kapodistrian University of Athens and other Research Centers and Universities in Greece. From these studies, AβC5-34 και AβC5-116 were found to interfere with the normal course of Aβ aggregation and the formation of typical Aβ fibrils, generating species with reduced binding to the neuronal surface and reduced toxicity *in vitro* and *in vivo*. Further, to showcase the generality of the approach, the NHRF team applied the same platform to identify efficient and highly specific misfolding rescuers of mutant Cu/Zn superoxide dismutase 1 (SOD1), a human enzyme associated with certain forms of amyotrophic lateral sclerosis, an ultimately fatal degenerative condition affecting motor neurons.

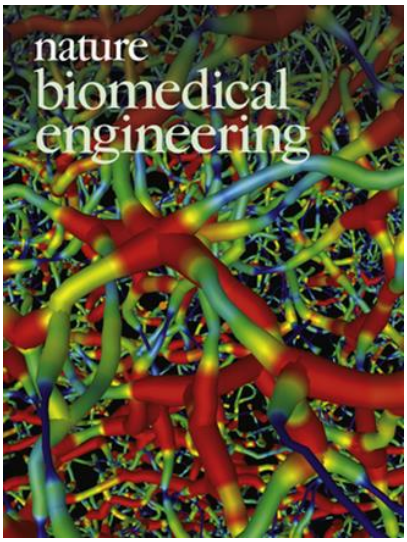
This newly developed technology offers a number of crucial advantages. First, the entire chemical library production and screening process is carried out in a simple bacterial host and, thus, its simplicity and speed are unprecedented. Second, it allows the investigation of previously untested chemical libraries with greatly expanded diversities, thus increasing the chances for identifying molecules with the desired biological properties and, at the same time, without prior intellectual property restrictions. Since the capabilities of this system in generating expanded chemical diversity are limited only by the transformation efficiency of *E. coli* cells, it can allow the production and screening of libraries with sizes ranging from tens of millions of members, such as the one investigated here, all the way up to tens of billions. Third, screening for bioactivity is carried out in a fully unbiased manner without requiring structural information about the targeted proteins, hypotheses about possible binding sites, or access to purified protein preparations. Finally, but very importantly, this new approach is generalizable and can be applied broadly for the discovery of macrocyclic rescuers of protein misfolding for a variety of disease-related targets.

In a "News & Views" commentary in *Nature Biomedical Engineering*, Prof. Frederic Rousseau from KU Leuven in Belgium points out that "The work by Skretas and colleagues is an example of the power of unbiased, high-throughput approaches that are also simple and robust. In neurodegenerative diseases, and in amyloid diseases in general, structural information on (early) toxic aggregates is scarce or non-existent, making modelling-based approaches difficult or impossible. In contrast, approaches such as that of Skretas and colleagues do not require any structural, or even mechanistic, knowledge, but are based merely on an intracellular assay for the desired effect", and concludes by stating that "The emergence of screening methods such as that presented by Skretas and colleagues … provide exciting opportunities for the discovery of new therapeutic molecules for these diseases, and for the development of strategies that

stabilize folded protein conformations or that inhibit the kinetics of aggregation of misfolded proteins".

This work has already resulted in two (2) PCT patent applications and has received five (5) awards for best presentation in international conferences in Greece and abroad, including the "EMBO Conference on Molecular chaperones: From molecules to cells and misfolding diseases" (http://events.embo.org/15-chaperone/) and the "3rd Symposium on Non-Globular Proteins in Molecular Pathophysiology" (http://ngp-net17.saske.sk/). Finally, NHRF researchers are currently performing preclinical development of the discovered molecules and are pursuing their commercial exploitation.

Reference:



"An integrated bacterial system for the discovery of chemical rescuers of disease-associated protein misfolding"
Ilias Matis, Dafni Chrysanthi Delivoria, Barbara Mavroidi, Nikoletta Papaevgeniou, Stefania Panoutsou, Stamatia Bellou, Konstantinos D. Papavasileiou, Zacharoula I. Linardaki, Alexandra V. Stavropoulou, Kostas Vekrellis, Nikos Boukos, Fragiskos N. Kolisis, Efstathios S. Gonos, Marigoula Margarity, Manthos G. Papadopoulos, Spiros Efthimiopoulos, Maria Pelecanou, Niki Chondrogianni, Georgios Skretas*
Nature Biomedical Engineering. 1 838-852 (2017)
DOI: 10.1038/s41551-017-0144-3
https://www.nature.com/articles/s41551-017-0144-3

News & Views commentary:
"Identifying rescuers of misfolding"
Tobias Langenberg, Joost Schymkowitz & Frederic Rousseau
Nature Biomedical Engineering. 1 782-783 (2017)
https://www.nature.com/articles/s41551-017-0149-y

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