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Edited by JOACHIM VON BRAUN
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TRANSFORMATIVE ROLES OF SCIENCE IN SOCIETY: FROM EMERGING BASIC SCIENCE TOWARD SOLUTIONS FOR PEOPLE'S WELLBEING

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"Today, both the evolution of society and scientific changes are taking place ever more rapidly, each following the other. It is important that the Pontifical Academy of Sciences consider how these interconnected changes require a wise and responsible commitment on the part of the entire scientific community. The splendid ivory tower security of early modern times has given way, in many, to a salutary unrest, for which today's scientists are more easily open to religious values and can glimpse, beyond the achievements of science, the richness of the spiritual world of peoples and the light of divine transcendence. The scientific community is a part of society, and must not be considered separate and independent; indeed, it is called to serve the human family and its integral development".

Pope Francis,
Address to the Pontifical Academy of Sciences,
12 November 2018

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Contemporary Challenges in Medical Usage of Antibiotics

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Introduction

The severe increase in antibiotic resistance and cross-resistance among many pathogenic bacterial strains[1] presents a significant health threat.[2] The marginal attention to severances of the results from antibiotics usage led to a rapid increase in the appearance of multi-drug resistant pathogenic bacterial strains.[3] Moreover, the extremely slow progress (actually negligible) in developing new antibiotics by pharma companies worldwide causes extreme contemporary issues.[4] Consequently, it seems that along with the traditional attempts to improve current antibiotics and the intensive screening for additional natural compounds, this field should undergo substantial conceptual revision. For example, the common preference for broad-spectrum antibiotics should be challenged, as it triggers the development of antibiotic resistance in a large variety of bacteria, including those that are not involved in the particular disease. In addition, many antibiotics do not distinguish between pathogens and some of the bacteria of the microbiome may thus alter the microbiome population.[5] Furthermore, most antibiotics are chemically substituted non-digestible and non-biodegradable small molecules that upon leaving the patient's body and reaching the environment, may escape the purification systems and cause ecological and environmental complications,[6] which in turn contribute to further resistance development. Here we describe our studies towards reducing and/or controlling antibiotics resistance, focusing on the identification of species-specific and environmentally-friendly protein synthesis inhibitors, which may be used as lead compounds for the development of novel drugs.

Expected Lower Rate of Resistance

The ribosome is the biological macromolecule responsible for protein synthesis according to the genetic code in all the living cells. In prokaryotes it consists of two ribosomal subunits, namely the large ribosomal subunit,

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called 50S (according to its sedimentation coefficient), and the small ribosomal subunit, or 30S (Fig. 1).

Owing to the vital role played by the ribosomes, many antibiotics target them.[8] Most ribosomal antibiotics obstruct the key ribosomal functions, namely decoding and peptide bond formation. As these sites are rather limited in number and chemical options, we initiated studies, based on our previous attempts to identify particular ribosomal structural motifs,[9] aiming at the development of novel antibiotics. Particularly we focus on "pathogen-specific antibiotics", in contrast to the current preference of broad range antibiotics. For this aim, we identified species-specific ribosomal structural motifs that may be exploited for the design of novel antibiotics of controllable chemistry.

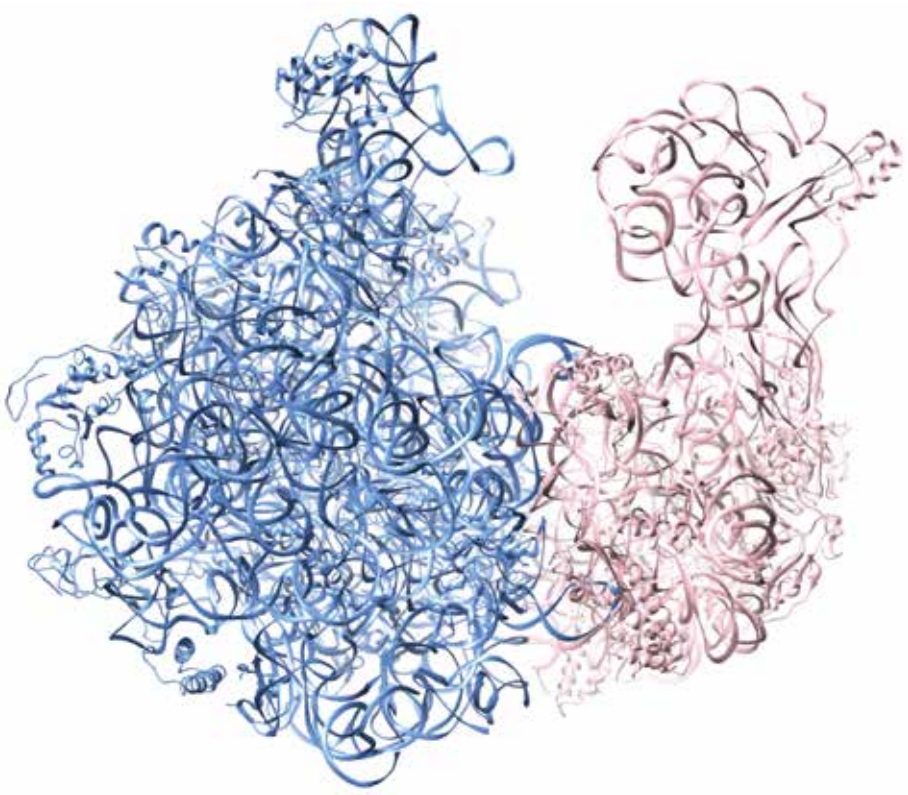

Figure 1. The skeleton of the ribosome from Staphylococcus aureus:[7] the large ribosomal subunit is represented in blue, the small ribosomal subunit in pink.

In principle, for each bacteria several such sites could be identified, and for each of these sites a specific matching molecule could be designed as a lead compound. Hence, for each pathogen we will have in hand a collection of lead compounds, each of which can be further developed into an antibiotic drug. These are planned to be used one at the time. Thus, the usage of species-specific instead of broad range antibiotics, alongside the expected variety of these antibiotics, which can be used consequently, are bound to lead to a slower pace of resistance appearance.

We are focusing on surface exposed sites, with chemical structure and composition allowing the design of matching compounds with favorable chemistry. These sites were discovered mainly by comparing the sequences and the structures of the large ribosomal subunit of the pathogenic bac-

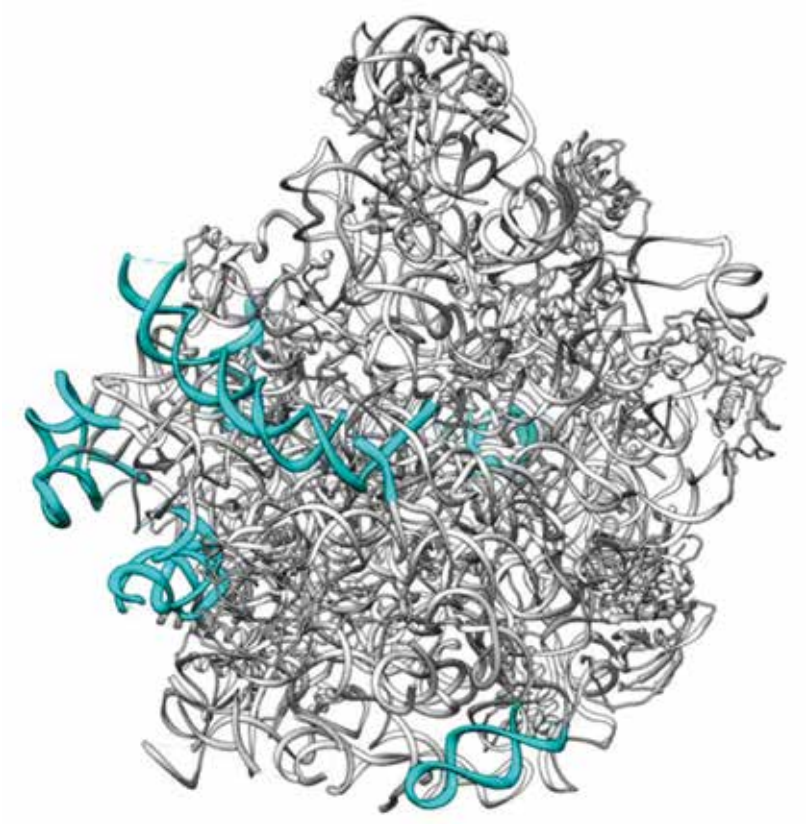

Figure 2. The skeleton of the large ribosomal subunit from Staphylococcus aureus.[10] Potential target sites for antisense therapy are shown in cyan.

teria of interest to those of other non-pathogenic bacteria. Our approach became feasible thanks to the recent year's availability of cryo-electron microscopy for the determination of high-resolution structures of large biomolecules like the ribosomes. Once the explicit pathogen unique sites were identified, solvent exposed regions of rRNA (Fig. 2) were selected. These were targeted by antisense oligonucleotides in a cell free *in vitro* transcription-translation activity assay, using luciferase as reporter gene to check protein synthesis inhibition.

The interactions between the inhibiting antisense oligonucleotides and their designed targets were then assessed by running fluorescein labelled versions of the oligonucleotides in an agarose gel, along with both the ribosomal subunits from the bacteria of interest, using a fluorescein labelled Shine-Dalgarno sequence[11] as positive control. In this way we observed selective binding of our designed antisense oligonucleotides to the large ribosomal subunit. These interactions, paired with a selective binding of the Shine-Dalgarno sequence to the small ribosomal subunit, represent a proof that the designed sequences are indeed binding to their predicted target sites.

Conclusions

Our studies are leading towards the design of "pathogen-specific antibiotics", in contrast to the current preference of broad-spectrum antibiotics usage. The discovery of the unique species target that can lead to antibiotics that will be used sequentially, alongside the speeding up of the procedures for the identification of the actual clinical pathogen (by a few companies) open new, untraditional path towards species specific antibiotics, of which sequential application should reduce and slow down the appearance of resistance.

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