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Evolution of *Staphylococcus aureus*

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Importance of mobile gene on bacterial virulence and

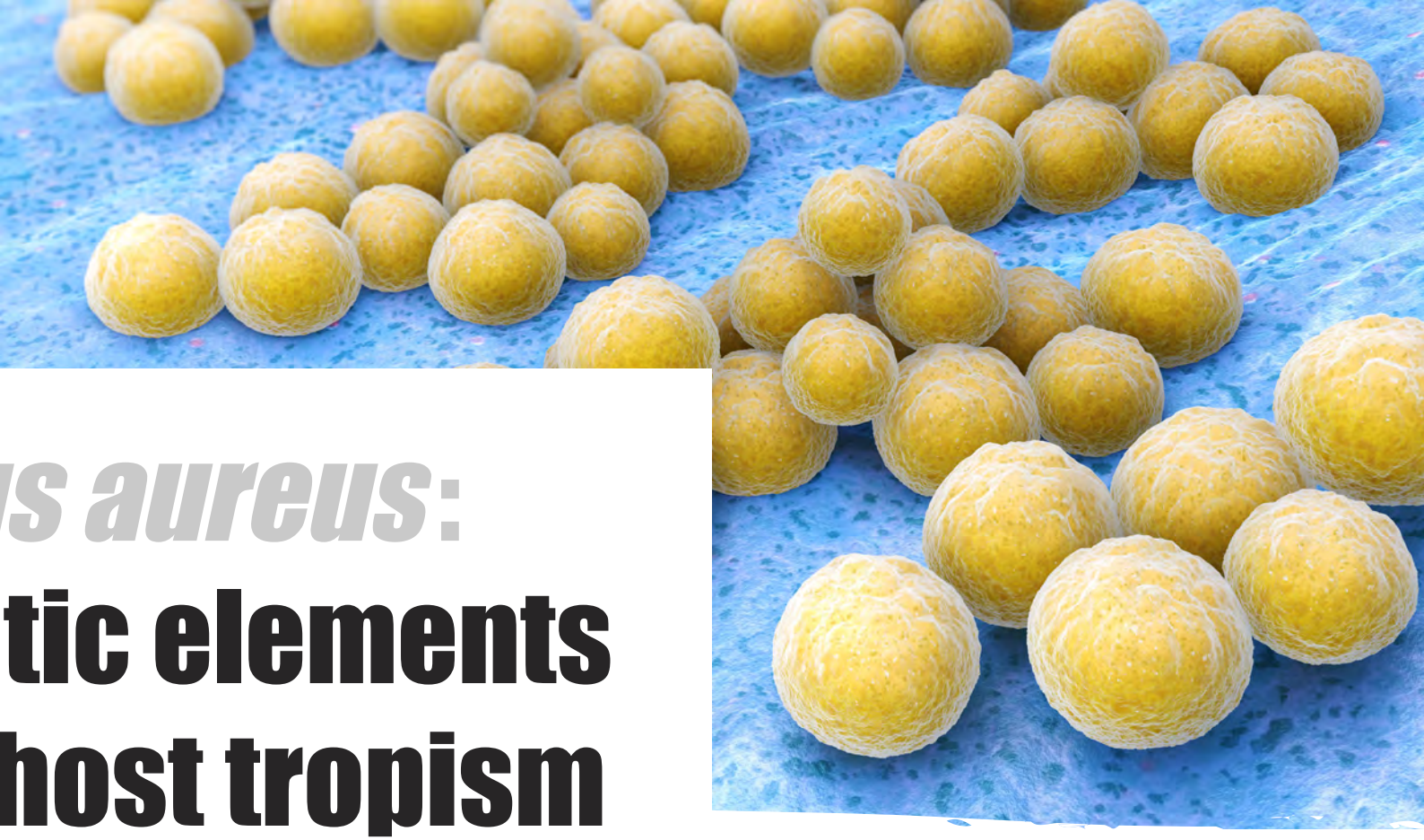
Introduction

For many decades, the clinical challenge due to *Staphylococcus aureus* infections in the hospital, community, and also in veterinary medicine has led to intensive investigations. While *S. aureus* can colonize 20-30% of the general population without causing any clinical manifestation, it is also capable of causing a wide spectrum of diseases in humans ranging from benign skin infections, through more serious diseases such as food poisoning, to life-threatening infections such as endocarditis, necrotizing pneumonia, osteomyelitis or septicemia. In milk-producing ruminants, mastitis is the major cause of economic loss worldwide and a major concern in milk transformation. Mastitis is currently hard to tackle in dairy cows and goats as this infection is refractory to antibiotic treatment in part due to the anatomic barrier and an aberrant immune response. In ewes in particular, severity of mastitis may vary from subclinical to lethal gangrenous diseases.

The versatility of this pathogen could be explained by different adaptative strategies and virulence properties. For instance *S. aureus* is capable to survive in various environmental conditions; acidic, oxidative, high temperature variations, as well as in nutrient-limited medium. In addition, *S. aureus* show particular capacity to resist to the presence of chemicals including antibiotics or antiseptic molecules. Some of the properties required to resist to these different conditions are intrinsic to the bacterium but others require adaptation and are linked to genomic evolution, in particular the acquisition of nucleic acid from various origins. Our purpose will be to evaluate the different strategies used by *S. aureus* to evolve and adapt its genome composition and content following natural events or after exposition to selective pressure.

Alteration of genome integrity

During the lifetime of a bacterial cell, various events contributed to modifications in genome content. DNA transposons are frequently found in clinical isolates. They are small linear mobile elements encoding generally for a limited number of genes and flanked by insertion sequences. These elements are able to jump within the bacterial genome. The integration could result in the disruption of a coding sequence but also bring new genes to the transposed bacteria such as antibiotic resistance genes. Plasmids are generally larger in



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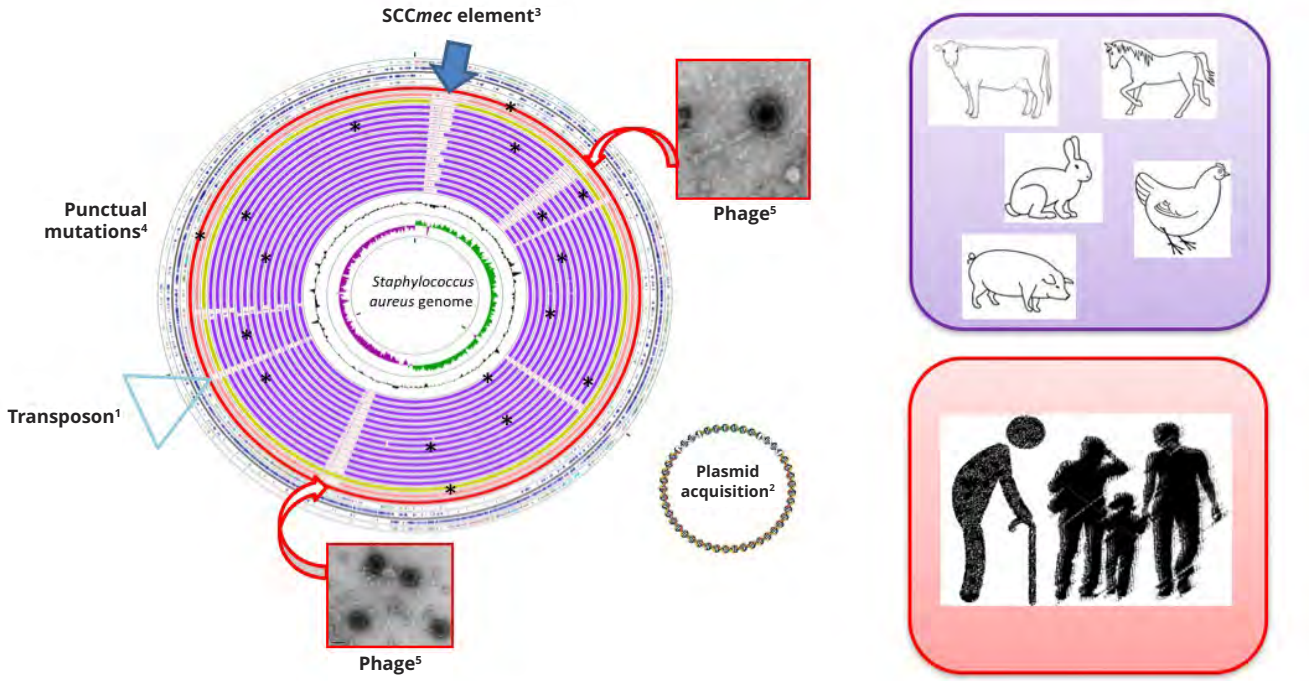
term of size and harbour frequently dozen of genes encoding potentially for antibiotic or heavy metal resistance. Most of the time, plasmids found in clinical isolates are circular autonomous molecules, able to replicate in the cytoplasm of the bacterial cells. Finally, lysogenic or temperate phages are also important partners of clinical isolates. Numerous infections are due to toxins encoded by bacteriophages (e.g. diphtheria toxin gene harboured by *Corynebacterium diphtheria* or scarlet fever toxin harboured by *Streptococcus pyogenes*).

In the clinic or in the environment, the frequency of genomic alterations is difficult to predict and is dependent on bacterial density and lifestyle. Considering populations of *S. aureus* strains and particularly of MRSA (methicillin resistant *Staphylococcus aureus*) – present in the clinic, almost all isolates showed in their genome traces of transposon, bacteriophage insertion or plasmids either residing autonomously in cellular cytoplasm or integrated in the bacterial chromosome. Note however that the most precious good for all cells is the protection of cellular integrity and particularly the protection of its genetic patrimony. This protection is the role of restriction modification systems. These systems are able to sense foreign DNA through specific biochemical motives and to decide to degrade this material with dedicated deoxyribonuclease. These systems are essential to protect integrity of the bacterial genome but in clinical isolates, non-functional restriction modification systems are not rare. In theory, acquisition of foreign material in deficient bacteria should increase genome plasticity.

The life of a bacterial cell

In the environment, bacteria from various species are in close contact and could exchange information, nutrients and genetic material. Bacterial death results in cellular envelop disruption and release of bacterial chromosome. These nucleic acids could be integrated by bacterium yielding to the acquisition of new genes. This process is named natural transformation. Considering bacterial populations, numerous mobile genetic elements could contribute to genome evolution. Plasmids, transposons or prophages represent efficient processes for the acquisition of new genomic features. In *S. aureus*, plasmids are frequently observed in clinical strains and encode generally for antimicrobial resistance determinants. *S. aureus* is also able to produce a plethora of toxins

Evolution of *Staphylococcus aureus* through integration of mobile genetic elements modulates baterial virulence and modifies host tropism



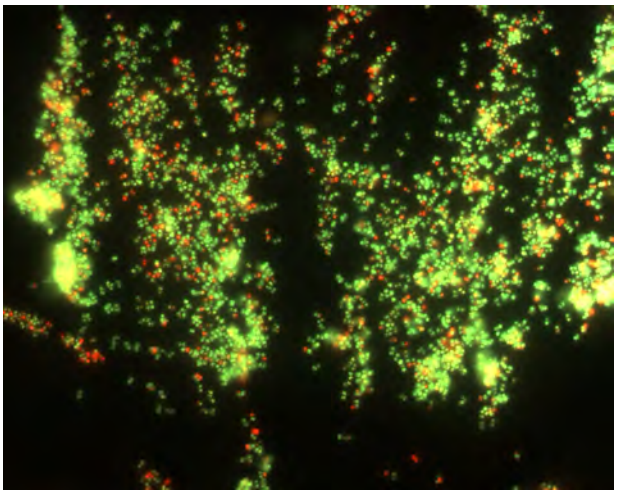
Pathways driving the evolution. Each circle corresponds to the circularized genome of a *Staphylococcus aureus* strain. The purple circles are genomes of animal colonizers only able to reside in animals (purple square) but not found in human (red square). During its lifetime the colonizer strain will be exposed to DNA transposons (1) often encoding resistance genes, acquisition of plasmid DNA (2) containing metabolic or resistance genes, to the integration of the SCC*mec* element (3) containing the genetic basis of methicillin resistance and will accumulate some punctual mutations (4) symbolized by * on the circular genomes. In our works, we showed that following these potential events triggering alteration of the genome integrity of the original strain, the ancestral susceptible colonizer strain remains a colonizer of animal but is now resistant to several antibiotics. It is only after integration of prophage nucleic acid (5) that the strain changes its tropism for host and is now able to infect human (red circular genome infecting human).

contributing to its virulence such as toxic shock syndrome toxin, Penton Valentin leucocidin, exfoliatin toxins A and B and more than 20 enterotoxins which are generally acquired through acquisition of integrative elements (e.g. lysogen bacteriophages).

S. aureus is a common bacterium able to multiply and survive independently, thus, this microorganism is not an obligatory parasite. In laboratory, in rich medium and in optimal conditions, *S. aureus* population density doubles every 30 minutes. It means that all the bacterial compounds or molecules could be duplicated in 30 minutes to generate a daughter cell "identical" to its mother. This phenomenon requires billions of enzymatic and chemical reactions in a limited times catalysed by the 2800 genes present in the bacterial genome. Even if protection mechanisms exist, punctual errors are possible and mutation occurs. This type of mutation appears spontaneously more frequently in non-coding sequences but could also yield to the modification of the sequence of a protein with unpredictable consequences. Note that if a mutation occurs in a vital gene, the cell could be non-viable. On the opposite, a mutation appearing in a target of an antimicrobial molecule could result in an increase resistance against this molecule. To

illustrate this evolution process, different groups observed emergence of spontaneous mutations in metabolic pathways yielding to optimization of the utilization of carbon source present in the growth medium. Another example is the mutation of a single base at specific location among the 2.8 million bases of the chromosome that triggers resistance against specific classes of antimicrobials such as quinolones or mupirocin. My group studied this phenomenon recently in a variety of clinical isolates of MRSA. We incubated MRSA strains from 10 different lineages frequently found in human clinic, in the presence of low concentrations of mupirocin. Typically, the working concentrations were sufficient to alter the growth rate of the bacteria but not sufficient to kill cells. Concentrations were increased, generally by 50% after 48h of incubation until reaching lethal levels. Then, DNA of strains recovered in the medium with highest concentrations allowing growth, were purified for sequencing purposes. In almost all strains showing increase level of resistance, we were able to identify mutations at specific site of the *ileS* gene, the target gene conditioning the resistance against mupirocin. In another study, we performed similar incubations of MRSA strains with biocides used for patient decolonization (e.g. polyhexanide and chlorhexidine). Surprisingly we observed in bacteria showing reduced susceptibility to the biocide, emergence of punctual mutations in a gene (*mprF*) known to mediate the resistance against daptomycin; an antibiotic used in the treatment of MRSA infection. This category of resistances, induced by the presence of antimicrobial is then dependent on the selection pressure.

Numerous antibiotic resistances are related to the acquisition of a specific gene of resistance. It is the case for numerous molecules currently used in the human clinic. For example, shortly after the introduction of penicillin in clinic, penicillin-resistant bacteria were identified. These resistant organisms had acquired a gene encoding for an enzyme conditioning the degradation of penicillin; a beta-lactamase. The resistance against methicillin is encoded by a mobile element; the SCC*mec* locus inserted at a specific site in the genome of the bacterium (see also figure legend on page 4). This element contains the gene *mecA*, responsible for the resistance against methicillin. Cyclines and glycopeptides are also families of molecules currently used in human clinic. Resistance genes are known against these families of drugs and different studies about the mechanisms triggered by these genes showed that they contribute to alter the structure of the target of the antibiotic (the ribosome and the bacterial cell wall, respectively). The question of the origin of these genes is of outstanding importance. Most of antibiotics used to treat infectious diseases are natural molecules produced by bacteria or fungi to limit competition for nutrients and protect their ecological



Glass surface covered by *S. aureus* biofilm. Fluorescence microscopy image showing bacterial cells that are actively multiplying onto the surface of glass lenses in a 3D structure. The picture shows a low content in dead cells (red coloured by propidium iodide) and a vast majority of multiplying cells (green, coloured by SYTO dye).

niche. The fact that an organism is able to produce a molecule able to kill cells implies that this cell is able to resist to the effect of the killing agent. It is mandatory that the producer organisms harboured the corresponding resistance gene. It is then easily conceivable that antibiotic producer cells represent the potential reservoir of resistance genes for other bacterial cells. The concept that resistance genes were present in the nature long time before the utilisation of antibiotics to treat infections has been demonstrated in the past in a fascinating study where the authors discovered numerous resistance genes in analysing permafrost sediments (Antibiotic resistance is ancient. Nature 477, 457–461).

"Some of the properties required to resist to these different conditions are intrinsic to the bacterium but others require adaptation and are linked to genomic evolution, in particular the acquisition of nucleic acid from various origins. Our purpose will be to evaluate the different strategies used by *S. aureus* to evolve and adapt its genome composition and content following natural events or after exposition to selective pressure."

In conclusion of these different observations, we showed that resistance against antimicrobials could result to exposition to the drug, or to the acquisition from another bacterial cell of a specific gene allowing resistance, thus following exposition to selection pressure. The most famous example was the introduction of the methicillin (a semi-synthetic penicillin resistant to β-lactamases) in human medicine in 1960. Only 6 months later, the isolation of the first MRSA was reported. The resistance was acquired through the integration of a large genomic element containing the methicillin resistance determinant as well as other resistance determinants, yielding to multi-resistant strains of *S. aureus* which are difficult to eradicate. We observed the mobility of the SCC*mec* element *in vivo* in our hospital in a patient initially identified as a MSSA carrier over several months. Further assays indicated a positive result for MRSA and the patient was then decolonized for MRSA by treatment with topical agent and biocide (mupirocin and chlorhexidine). Subsequent screening showed that the patient was still colonized with MSSA only. Molecular assay allowed us to demonstrate that the 3 strains (initial MSSA, MRSA and the last MSSA) were clonal, indicating the mobility of the SCC*mec* element in this patient. A very recent example of evolution regarding bacterial tropism has been reported in the veterinary clinic. *S. aureus* strains infecting poultry showed increased capacity to grow at 42°C and to alter avian cells reflecting a "human-to-poultry host transition". Note that this temperature in generally not compatible with growth of strains from the human clinic. This observation implies that most of essential proteins evolved through structure showing increase efficiency at 42°C, which is not the optimal growth temperature of the species.

My group is also active in this domain of adaptation of animal *S. aureus* strains to the human clinics. Originally detected exclusively in pig farmers in Europe, *S. aureus* belonging to the clonal complex 398 (CC398) lineage has become a worldwide threat associated with livestock, their human contacts and food products. During collaboration work with Doctor Nathalie van Der Mee-Marquet (University Hospital of Tours, France), who performed an active bloodstream surveillance program since 2000, we observed constant increase in the prevalence of sequence type 398 (ST398) in patients living in animal-free environments; from 0% in 2007 to 15% of *S. aureus* isolates responsible for severe infections in 2015. Basically, this surveil-

lance program allowed us to identify: i) ancestral ST398 strain only able to colonize animal, ii) pre-evolved ST398 strains able to infect animal, iii) evolved strains infecting or colonizing human in contact with animals and iv) ST398 isolates infecting human even without contact with animal. Important efforts were deployed using whole genome sequencing approach on these different populations of isolates. Analysis of the genomes of these isolates showed that each population contained specific prophage content. Whereas the ancestral isolate associated with animal are devoid of prophage, emerging clades are characterized by the presence of Φ3 prophage variants that encode two immune-modulating proteins that alter or prevent chemotaxis, phagocytosis and killing of *S. aureus* by human neutrophils. Recently, we mobilized prophages from virulent strains and introduced them in ancestral non-human pathogenic isolate. Our experiments clearly showed acquisition of new features, potentially mediating the virulence of the bacterium, accompanying acquisition of phages. In vitro experiments showed that strains containing prophages have increased capacity to interact with human extracellular matrix proteins and increased ability to penetrate into the cytoplasm of non-phagocytic cells. Even if *S. aureus* is not recognized as an intracellular bacterium, this capacity allows the bacterium to survive in a protected niche, hidden from cellular or humoral defences. In addition, in an experimental model of infectious endocarditis, we showed that ST398 isolates containing prophages were more prone to infect cardiac tissue and to multiply within cardiac vegetations, yielding to more severe infection than the prophage-free parental strains. Phages serve as a driving force in bacterial pathogenesis, contributing both to the evolution of bacterial hosts through gene transfer, and to bacterial pathogenesis at the time of infection. Temperate bacteriophages play an important role in the pathogenicity and cellular tropism of *S. aureus*.

Conclusion

Even if preservation of the genetic patrimony is essential for all organisms and cells are equipped with systems dedicated to its protection, genomes are dynamic structures undergoing modifications. Some modifications are discreet (punctual) whereas other events are more important with the transfer of large genetic elements. Ability to resist to environmental stresses, including the presence of antimicrobial drugs is dependent on natural adaptation or direct acquisition of new genomic elements. Natural adaptation could be a "try and error" phenomena that results in bringing advantages and is more frequently observed in the presence of selection pressure. Transfer of larger element encoding for numerous genes is able to modify important phenotypic properties of the bacterial cells and modulate its virulence as well as its host tropism.

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Supporting basic research in Switzerland

Basic research is a key area that the Swiss National Science Foundation (SNSF) promotes and funds, as Open Access Government's Ciara Ruane outlines...

The Swiss National Science Foundation (SNSF) primarily supports basic research: i.e., research that is not tied to a specific institution or industry, but seeks to improve knowledge overall. The SNSF describes the projects they fund as 'use-inspired', which means research that aims to provide practical solutions to problems.

Use-Inspired Basic Research (UIBR) differs from standard basic research in a number of ways. The SNSF defines it as dual-purpose, with an aim to "solve or illuminate one or several practical problems, as well as advancing science". Applications marked UIBR face a more selective process and are less likely to be successful than others. Last year this approach was evaluated by an independent consulting firm called Technopolis, focusing on their definition of UIBR. The report stated that the introduction of the UIBR category in 2011 had allowed the SNSF to broaden the scope of their research and did not require an overhaul. However, it recommended that the category be defined 'more clearly'. They also stated that the reduced success rate of UIBR applications could be attributed to the complexity of the dual-purpose approach. It also suggested more diverse selection panels and a greater emphasis on the 'broader impact' criteria outlined under UIBR.

Encouraging young people into research

The SNSF places a great deal of emphasis on encouraging young people to pursue careers in research and development. They outlined a 2013-2016 action plan to best achieve this. They emphasise a 'career friendly' approach, removing obstacles and restrictions that make it difficult for young people to secure a permanent role below the level of a professorship. The aim outlined was to increase doctoral student salaries by 7%, increase the potential for promotion, and provide support for doctorate students with families. The '120% model' is aimed at those who have childcare obligations impacting their work, and allows them to work reduced hours with the chance to apply for childcare funding.

"The SNSF places a great deal of emphasis on encouraging young people to pursue careers in research and development."

The SNFS has different funding programmes available for researchers at different stages of their career. They have a guide on which programme is best suited to the individual, detailing the level of study, the nature of the test, and what resources are required. The overall aim is to provide complete flexibility for applicants, allowing people in a multitude of different circumstances to pursue a career in research and development. They also have international study schemes available, through partnerships with countries in Asia and Eastern

Europe. They hope this allows young researchers the opportunity to study in a new environment and build an international network for Switzerland within the scientific community.

Ethical practices

The SNFS makes a strong point of reinforcing ethical practices. In 2014 they published a document detailing their use of animal testing. The SNFS follows the guidelines laid out by relevant authorities and does not get personally involved with decisions on the ethics of animal testing in individual research projects. The report states that animal testing makes up for a small part of the research process, and currently there is no effective alternative available for most experiments. However, they state that animal suffering "must be kept to a minimum", and researchers often use alternative models such as cell structures. They state that they welcome debate on the issue, but distance themselves from 'misinformation'.

Gender equality

The SNFS takes a firm stance in favour of gender equality. Their statement on the issue outlines a 'gender neutral' atmosphere they hope to create, with a zero-tolerance policy towards any form of prejudice. They have a number of legislative actions aimed to enforce this goal. They have a gender equality grant, specifically targeted at young women researchers, a 'non-discriminatory pay system', work-at-home options to balance family life, and carries out regular evaluations to ensure equality is being maintained. These efforts are ongoing, and the SNFS places responsibility on the highest levels of the organisation. This goes alongside their aim of encouraging all young researchers and creating a more forward-thinking research community.

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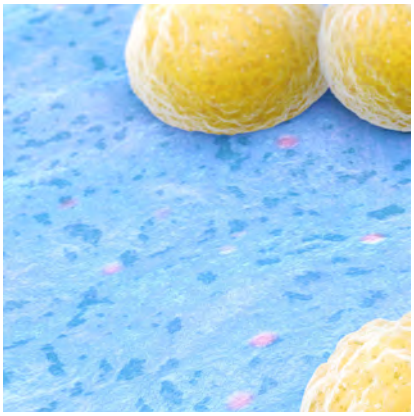
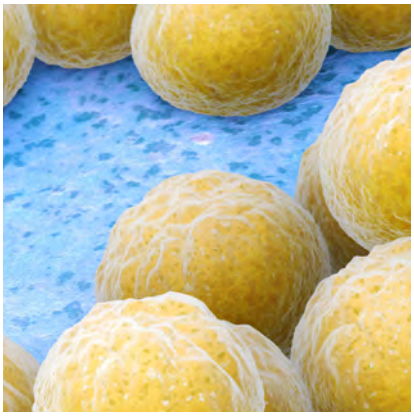
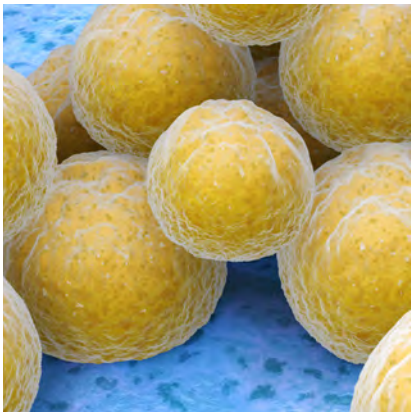
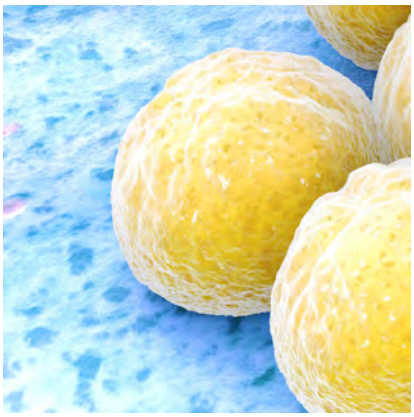
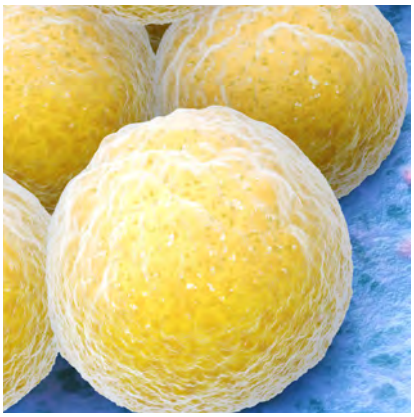
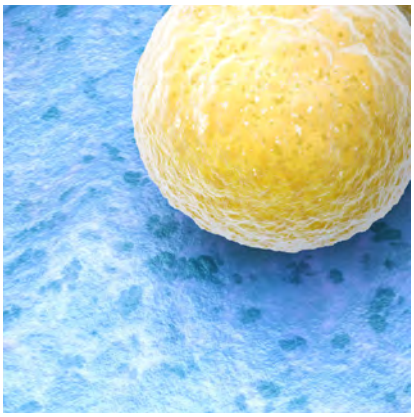

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