

Genomics and synthetic biology as a viable option to intensify sustainable use of biodiversity

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The Amazon basin is an area of mega-biodiversity¹. Different models have been proposed¹⁻⁸ for the establishment of an effective conservation policy, increasing sustainability and adding value for biodiversity. Over the years, the Amazon region has been caught in a vicious circle that involves deforestation and falling employment. There is a hypothesis that links a reduction in deforestation and an increase in governance with the higher employment and income that are associated with the development of various processes and products, and this has been extolled as a virtuous development circle⁷. Generating carbon credits has also been suggested as a viable development strategy for the region^{8,9}. However, the real value of biodiversity directed to the potential product development remains unknown and the estimates are speculative. Evidence-based studies of the Amazon region should be urgently intensified and expanded. These should be based on the evaluation and potential development of sustainable high-value added products. Currently, a broad spectrum of technologies from genomics to synthetic biology is available, and these permit the collection, manipulation and effective evaluation of countless organisms, metabolic pathways and molecules that exist as potential products of a large, biodiverse ecosystem. Recently, a general development cycle has been proposed for products a given product arising from the recombinant DNA technology associated with agri-business¹⁰⁻¹¹. Basically, the development cycle establishes the appropriate nomenclature and lists the interactive stages

involved, from the prospecting of candidate molecules through their characterisation, testing and deregulation. However, the potential utilisation of synthetic biology and recombinant DNA technology directed to the aggregation of biodiversity value is illustrated here (Fig. 1). This figure shows possible behaviours of the dependence of the associated values as a function of time, for an arbitrary period of 10 years. Ideally, the gain in knowledge from the ongoing studies will lead to diminishing risks of product failure and a consequent rise in the associated and expected economical values. However, in a real situation, problems encountered during research may render exploitation of the natural resources impractical. Therefore, the inverse picture, in which the value decreases over time, may also occur (Fig. 2).

The development of advanced science-based products is directly correlated with the intensive utilisation of knowledge and high technological standards. This indicates that the utilisation of genomics and synthetic biology technologies in its full sense constitutes a viable option and a useful imperative tool for directing the sustainable use and development of high value-added products. From the genome to the creation of novel biological systems, metabolic pathways and molecules engineering^{12,13,14}, implies the collection of a few organisms (or parts of them) as a source of genetic raw material that allows prospecting, characterisation, gene expression, new pathways and genes arrangements and the necessary proof-of-concept studies (research to demonstrate the functional efficacy of a trait or technology in the target organism, to reduce the risk of product failure) aiming in a broad sense the improvement of human health and a sustainable use of biodiversity. Genomics and synthetic biology have opened the door for another breakthrough in science advancement. Making the use of genomics as a founded template on the chemical synthesis and assembly using recombinant DNA technology and synthetic biology allows us to manipulate and create novel metabolic pathways and molecules of choice. An example follows that describes one of the several positive possibilities commented above. Spider silk has been noted for its extraordinary physical and mechanical properties and has recently been recognised as a protein-based nanomaterial. It has had the ability to self-assemble into fibres consisting of antiparallel beta-sheets for more than 450

million years¹⁵. Spider silks are protein-based biopolymers secreted through specialised epithelial cells as concentrated soluble precursors of repetitive building blocks of primary protein structures. These biopolymers are highly flexible and exhibit extraordinary strength and toughness, comparable to high-performance synthetic fibres such as Kevlar. Despite the high potential of spider silk arising from its mechanical characteristics, the impossibility of domesticating spiders to produce fibres in sufficient quantities has led to the development of alternative strategies for the production of silk proteins using synthetic biology recombinant DNA technology¹⁶

To this end, we have conducted an ongoing initiative on the functional genome of Brazilian spider silk glands that studies evolution at the molecular level with the goal of developing novel biopolymers through synthetic biology¹⁷⁻¹⁹. The possibility of producing novel biodegradable materials with similar properties has motivated increased research on silk proteins (spidroins). Out of the genomics, different spidroin sequences produced by the major ampullate, minor ampullate, flagelliform and tubulliform silk glands from spiders were identified. The spiders, *Nephilengys cruentata*, *Avicularia juruensis* and *Parawixia bistriata*, were collected and prospected in the Brazilian biomes¹⁷⁻¹⁹. Drawing on genome and transcriptome data, we were able to design de novo proteins and produce synthetic spider-like fibres (Fig. 3).

Currently, different expression systems, such as bacteria²⁰, yeast²¹, plants²², and animals,²³ have been evaluated to define the most suitable system for producing synthetic spider-like proteins on a large scale at a lower cost. We have demonstrated the production of synthetic proteins that can form spider-like fibres in bacteria¹⁶ and soybean seeds (data not shown). It is expected that in addition to the potential for developing high value-added and innovative biomaterials, this work might also be used as a model that might fit the equation described in Figure 1.

In addition to the efforts made to conserve biodiversity, an effective, evidence-based method for estimating biodiversity value is necessary. The use of Genomics and synthetic biology may constitute an important tool and be a viable option for the prospection, evaluation and manipulation of biodiversity as

advocated as well as be useful for developing methods for sustainable use and the production of novel molecules. Biotechnology may well fit into the virtuous circle option. Due to the urgency and strategic importance of this subject, the evaluation model described here may contribute to induce the intensification and establishment of operative partnership between the public and private sectors, along with the necessary interactions with developed countries.

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References

1. Soares-Filho, B.S., Nepstad, D.C., Curran L.M., Cerqueira G.C., Garcia R.A., Ramos C.A.O, Voll E.I, McDonald A., Lefebvre P., Schlesinger P. Modelling conservation in the Amazon basin. *Nature* **440**, 520-523 (2006).
3. Peres, C.A. & Terborgh, J. W. Amazonian nature reserves: an analysis of the defensibility status of existing conservation units and design criteria for the future. *Conserv. Biol.* **9**, 34-46 (1995).
2. Merry, F.D., Amacher, G.S., Lima, E. & Nepstad, D.C. A risky forest policy in the Amazon? *Science* **299**, 21 (2003).
4. Gullison, R.E., Frumhoff, P. C., Canadell, J. G., Fieldm C.B., Nepstad, D.C., , Hayhoe, K., Avissar, R., Curran, L.M., Friedlingstein, P., Jones C.D., Nobre, C. Tropical Forests and Climate Policy. *Science* 316, 985-986 (2007).
5. Schwartzman, S., Moreira, A. G. & Nepstad, D. C. Rethinking tropical forest conservation: perils in parks. *Conserv. Biol.* **14**, 1351-1357 (2000).

6. Nepstad D., Schwartzman, B., Bamberger, B., Santilli, M., Ray, D., Schlesinger, P., Lefebvre, P., Alencar, A., Prinz, E., Fiske G., Rolla, A., Inhibition of Amazon Deforestation and Fire by Parks and Indigenous Lands. *Conserv. Biol.* **20**, 65-73 (2006).
7. Nepstad, D. *et al.* Frontier Governance in Amazonia. *Science* **295**, 629-631 (2002).
8. Nepstad, D., Sticker, C.M. & Almeida, O.T. Globalization of the Amazon Soy and Beef Industries: Opportunities for Conservation. *Conserv. Biol.*, **20**, 1595-1603 (2006).
9. Stern, N. *The Economics of Climate Change: the Stern Review* (Cambridge Univ. Press, Cambridge, 2006); www.hm-treasury.gov.uk/independent_reviews/stern_review_economics_climate_change/stern_review_report.cfm.
10. Mc Elroy, D. Valuing the product development cycle in agricultural biotechnology – what's in a name. *Nature Biotechnology* **22**, 817-822 (2004).
11. Mc Elroy, D. Sustaining agbiotechnology through lean times. *Nature Biotechnology* **21**, 996-1002 (2003).
12. Drew, E (2005) Foundations for engineering biology. *Nature Reviews* **438**:449-453
13. Keasling, J.D. (2008) Synthetic Biology for Synthetic Chemistry. *ACS Chem. Biol.* **3**:64–76.
14. Martin C,H,, Nielsen D. R., Solomon K.V., Prather L. J. (2009) Synthetic Metabolism: Engineering Biology at the Protein and Pathway Scales. *Cell* **16**:277-286

15. Lewis, R.V. Spider silk: Ancient ideas for new biomaterials. *Chem. Rev.* **106**, 3762-3774 (2006).
16. Teulé, F., Cooper, A. R., Furin, W. A., Bittencourt, D., Rech, E.L., Brooks, A., Lewis, R. V. (2009) A protocol for the production of recombinant spider silk-like proteins for artificial fiber spinning. *Nature Protocols*, **4**:341-355.
17. Rech, E.L. *et al.* Patent application. Silk proteins from the spiders *Nephylengys cruentata*, *Avicularia juruensis* and *Parawixia bistriata*, isolated from the Brazilian biodiversity. PI0701826-6.; http://v3.espacenet.com/searchResults?DB=EPODOC&submitted=true&locale=en_EP&AB=CRUENTATA&ST=quick&compact=false
18. Bittencourt, D., Souto, B.M., Verza, N.C., Vinecky F., Dittmar, K., Silva Jr., P.I., Andrade, A.C. , da Silva, F.R. , Lewis, R.V. Lewis, Rech, E.L. (2007) Spidroins from the Brazilian spider *Nephilengys cruentata* (Araneae: Nephilidae). *Comp. Bioch. and Physiol. Part B: Biochemistry and Molecular Biology* **147**:597-606.
19. Bittencourt D., Dittmar, K., Lewis, R.V., Rech, E.L. (2010) A MaSp2-like gene found in the Amazon mygalomorph spider *Avicularia juruensis*. *Comp. Bioch and Physiol., Part B: Biochemistry and Molecular Biology* **155**:419-426.
20. Fahnstock, S.R. & Irwin, S.L. Synthetic spider dragline silk proteins and their production in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* **47**, 23-32 (1997)
21. Fahnstock, S.R. & Bedzyk, L.A.,. Production of synthetic spider dragline silk protein in *Pichia pastoris*. *Appl. Microbiol. Biotechnol.* **47**, 33-39 (1997).
22. Scheller, J., Gurhuns, K.H., Grosse, F. & Conrad, U. Production of spider silk proteins in tobacco and potato. *Nature Biotech.* **19**, 573-577 (2001).

23. Lazaris, A. *et al.* Spider silk fibers spun from soluble recombinant silk produced in mammalian cells. *Science* **295**, 472-6(2002).

Figure 1. Biotechnology and biodiversity value aggregation. This figure shows possible outcomes for the dependence of the associated value as a function of time for an arbitrary period of 10 years. The graph depicts the function $v_n(x)=100(x/10)^n$ for varying values of n as an illustration for the various possible outcomes.

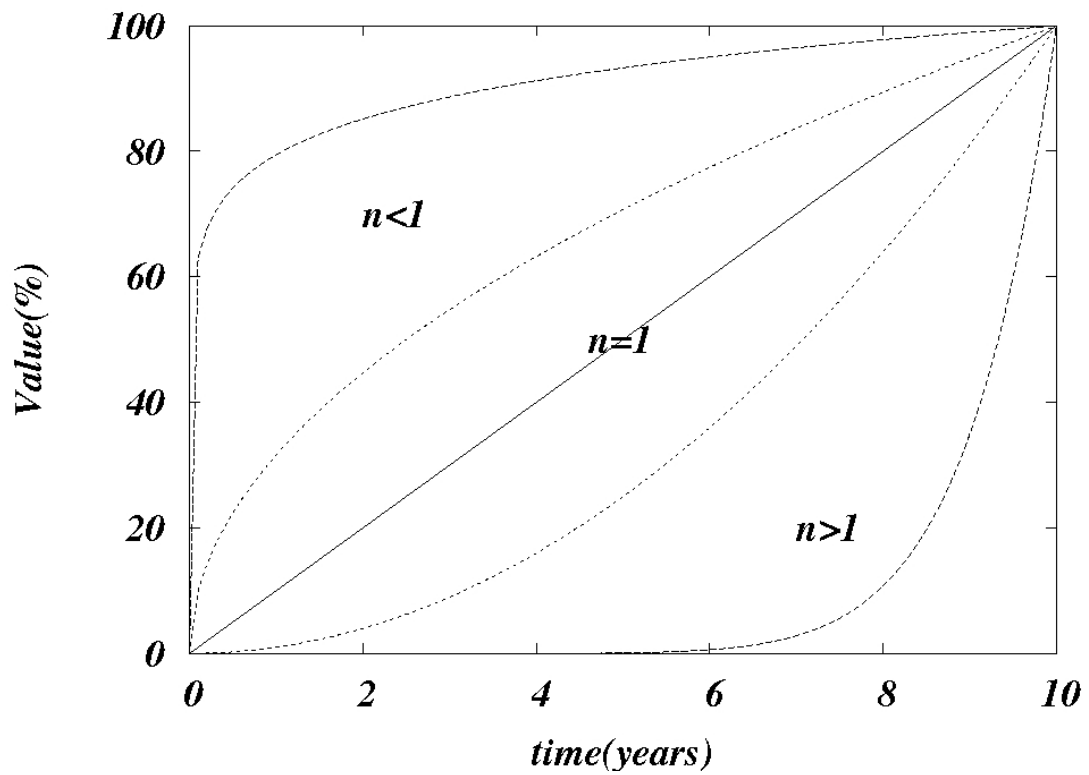


Figure 2. Biotechnology and biodiversity value aggregation. Due to problems during the research, the exploitation of a certain natural resource may prove impractical and therefore the associated value decreases with time. This figure shows possible outcomes for the dependence of the associated value as a function of time, for an arbitrary period of 10 years. The graph depicts the function $v_n(x)=100 -100(x/10)^n$ for varying values of n as an illustration for the various possible outcomes.

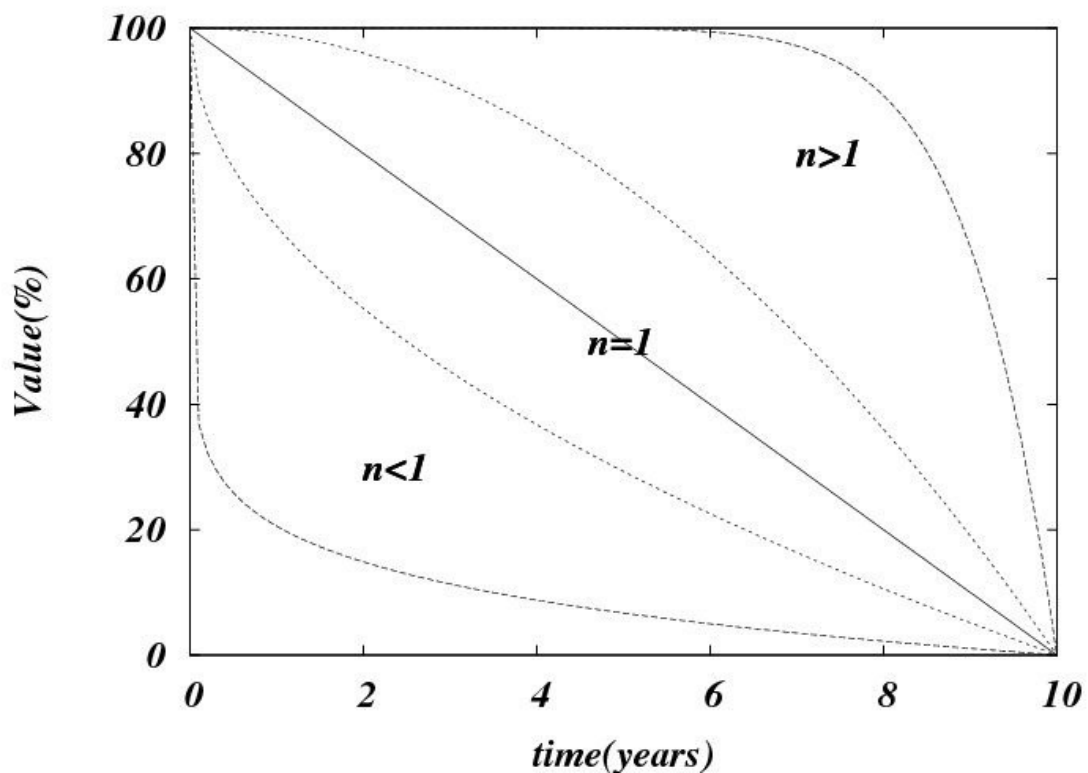


Figure 3. A representative atomic force microscopy image of the topographic surface of a synthetic spider silk-like fibre made from the synthetic recombinant protein¹⁷ MaSp 2 from *Parawixia bistrata*.

