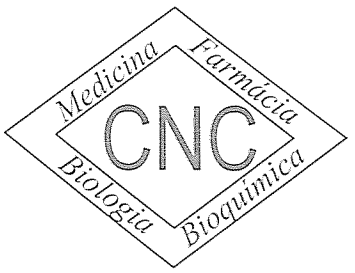

Centro de NeuroCiências e Biologia Celular

UNIVERSIDADE DE COIMBRA

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Yves Brun,
Systems Biology/Microbiology Faculty Search,
Department of Biology, Indiana University,
Jordan Hall 142, 1001 E 3rd St,
Bloomington IN 47405-7005

Coimbra, October 31, 2005

Dear Dr. Brun,

I am writing in support of Dr. Rui Alves' application for a Faculty Position in Systems Biology/ Microbiology at the Department of Biology and Biocomplexity Institute, Indiana University. I have informally co-supervised Dr. Alves' graduation ("licenciatura") thesis at the University of Lisbon (Portugal) in 1993-94, and I have been a member of the jury for his Ph. D. examination, at the same University, in 2000. We have overlapped at Dr. Michael Savageau's lab at the University of Michigan from 1998 to 2000, and we have an ongoing collaboration. Thus, I have a very good appreciation of Dr. Alves' qualities and research activities.

Dr. Rui Alves is a bright, enthusiastic, creative and very productive researcher. In his Ph. D. work, Dr. Alves developed a sampling method to compare systemic properties of ensembles of biological networks and applied this method to clarifying the design principles of various representative biochemical networks. Dr. Alves' original approach to the analysis of biochemical performance represented a major advance in the study of design principles of biological networks: it overcame important limitations of the traditional method of mathematically controlled comparisons and opened way to the analysis of more-complex networks. These advantages enabled Dr. Alves to gain important insights about the evolutionary design of unbranched biosynthetic pathways. Altogether, this 3-year doctoral research work originated five papers of which Dr. Alves is first author.

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At the post-doctoral level, Dr. Alves has invested strongly in the acquisition of a wide spectrum of genome analysis and computational biology skills, which he successfully applied in understanding important aspects of evolutionary design in bacteria and yeast. For instance, applying a whole-network approach he showed that retro-evolution of enzymes is more prevalent than had been thought. Recently, he demonstrated that the aminoacyl composition of proteins that participate in amino acid biosynthetic pathways in bacteria is strongly biased against the cognate aminoacid, and he showed that the response time and extent of de-repression of these pathways in response to amino acid depletion are important factors in the evolution of this outcome. We are currently collaborating in determining whether the oxidizability of amino acids is a significant selective pressure in determining surface amino acid composition of proteins.

Dr. Alves' creativity and willingness to interact with experimental microbiologists is also illustrated by his recent work on the predictive reconstruction of the iron-sulfur cluster assembly pathway of *Saccharomyces cerevisiae*, which I recently had the opportunity to review. This ambitious work, for which Dr. Alves provided the major intellectual input, cleverly integrates very disparate data, combining a diversity of bioinformatics and computational biology methodologies (phylogenetic analysis, homology and *ab initio* modeling of protein structure, biochemical systems analysis, etc.), to achieve a convincing reconstruction of a pathway that involves about a dozen proteins. While the proposed model is entirely consistent with the available experimental evidence, many of its features represent novel experimentally testable hypotheses. To my knowledge, such a well-supported large-scale predictive reconstruction of a biochemical pathway had never been achieved before.

I consider Dr. Rui Alves' standing in the field of Systems Biology well above average as compared to scholars at a similar academic level, and I have no reservations whatsoever about his high potential for a successful career at the Indiana University.

With kind regards,

Armindo Salvador
(Auxiliary investigator, CNC)

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Mr. Yves Brun
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October 29th, 2005

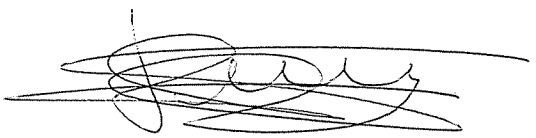
Sirs,

This is to inform you about Dr. Rui Alves, who has applied for a position at your institution. I know Dr. Rui Alves since about four years ago, when he arrived at the Department of Basic Medical Sciences of the University of Lleida, Spain, to work at the biostatistics group of Dr. Albert Sorribas. My group is interested in the effect of oxidative stress on cell growth and in the molecular systems involved in the regulation of protein redox state, using the yeast *Saccharomyces cerevisiae* as model organism. At this respect, among other projects we are studying the participation of the monothiol glutaredoxin Grx5 in the mitochondrial synthesis of iron/sulphur clusters. Immediately, I appreciated the scientific value of Dr. Alves not only when working on classical biostatistics problems, but mainly in modelling complex functional cell systems using genomic and proteomic tools. Therefore, I asked him for help in the theoretical analysis of iron/sulphur cluster biogenesis at the mitochondria. This is a complex problem in biology, since a large number of proteins participate in the process by physically and functionally interacting among them. However, the details of the process are still unknown. From the work of Dr. Alves, a predictive model for the mitochondrial process has been proposed, which opens new possibilities for directed bench experiments. It has resulted in two high standard publications. In addition, I am collaborating with Dr. Alves in the prediction of structure and interactions among other proteins related to oxidative stress, and have asked him for help in problems related with systems biology and bioinformatics that are continuously arising in my work. A third common publication has resulted from that collaboration, which addresses the comparative analysis of Grx5 and its bacterial orthologues. Therefore, I consider myself competent to inform on the scientific abilities of Dr. Alves and to know about his human interactions with laboratory fellows, which makes my opinion to be well founded.

As above indicated, Dr. Alves is a bright researcher with an excellent background in bioinformatics but also in basic biological sciences such as microbiology, biochemistry or

genetics. The interplay between the knowledge of specific biological processes and the application of systems biology concepts is extremely valuable in Dr. Alves. This allows him to perfectly understand the biological problems in order to apply the adequate bioinformatics/mathematical tools to address them. This expertise in biology is not commonly found in bioinformatics researchers, and therefore makes Dr. Alves especially valuable as a researcher in systems biology. Dr. Alves experience allows him to autonomously carry out the research projects, although he is also prone to collaborate with other researchers in areas requiring bioinformatics expertise. On the other hand, Dr. Alves is very active in the organization of common research discussions between research groups. In fact, shortly after arriving to this department, he organized a Journal Club that vividly promoted the participation of Ph.D. students in scientific discussions. This is only an example of his implication in common research activities at the whole department level, and also of his virtues as a person prone to collaborate with fellows and to help in the solution of any scientific problem which he is confronted with. In summary, he is an excellent researcher and a friendly person in the laboratory environment. Globally, I consider him among the top 5% of the post-doctoral researchers that I have known.

For all the above reasons I firmly recommend him for the applied position at Indiana University.



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