PAC research in Biology

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Abstract: In this paper possible applications of Perturbed Angular Correlations (PAC) technique in Biology are considered. Previous PAC experiments in biology are globally analyzed. All the work that appears in the literature has been organized in a few lines of research, just to make the analysis and discussion easy. The commonly used radioactive probes are listed and the experimental difficulties are analyzed. We also report applications of ^{181}Hf and ^{111}In isotopes in lifesciences other than their use in PAC. The possibility of extending these studies using the PAC technique is discussed.

Key words: perturbed angular correlation, ^{111}In, ^{181}Hf , proteins

1. Introduction

The Perturbed Angular Correlation (PAC) technique is very appropriate for studying the hyperfine interactions of radioactive atoms (the probes) in condensed matter.
PAC is a powerful method for characterizing nanoscopically ordered materials and has been applied to the study of the interactions between metallic probes and their surroundings. In adittion, the interaction between metallic atoms and biological systems has attracted the attention of the scientific community for a long time. Such an interest should not surprise since almost 40% of the proteins present in the human body interact with cofactors involving one or more essential metallic atoms including calcium, iron, zinc, cobalt, molybdenum, copper, etc. These proteins are involved in many essential physiological functions like redox reactions, transport, storage and transcription/ translation of genetic information. Moreover, several proteins are involved in the detoxification pathways for dangerous substances and interact with non-essential toxic atoms like mercury, chromium, arsenic, cadmium, gold, silver, lanthanides and actinides.

The history of the use of the PAC technique in biological systems started with the studies of motion and structure of biological macromolecules [1]. It was believed that PAC technique could gain usefulness as a labelling method, giving information concerning the localized behaviour and structure of a molecule near the labeled site [2]. The paper from R. Bauer [3] reported the first applications to understand the structural modifications induced by pH changes around Cd(II) probes in carbonic anhydrase. In this paper the authors developed the basis for the theoretical analysis using the Partial Quadrupole Interactions implemented in the Angular Overlap Model [4].

In the concluding remarks at the Groningen Conference of 1983, E. Bodenstedt recognized that the PAC applications to biological macromolecules make the method very attractive to biophysicists [5]. At the same time, the study of the atomic configurations around probe sites in biological systems, also both the cellular uptake

and metabolism of metals [6] and cellular stability and dynamics [7] were being studied by PAC.

The first review about PAC applications to Biology came from Bauer´s group in 1985 [8]. Other reviews on the applications of nuclear analytical techniques [9] and PAC [4; 10-13] to Biology appeared afterwards. The principal biological areas where the technique has been applied since the beginning and the most used PAC probes are presented in the form of bar graphs. Additionally, the applications of some PAC isotopes to biological systems other than the PAC use are reviewed. The aim of this study is to get a broader basis for new PAC applications to Biology. Several experiments, which are underway, are described.

2. Reported PAC applications to biological systems

There are about 120 papers concerning PAC applications to biological systems **(**PABS), most of which have been considered in this work. As it can be seen in Fig.1, the number of publications per five-year periods increased until the earliest nineties and is actually decreasing. We also reviewed which probes were used in these studies (Fig. 2). It can be observed that the most popular probes in PABS are ^{111m}Cd (36% of the total publications) and ^{111}In (32%). ^{111m}Cd, essentially used in studies of metalloproteins because it can easily substitute for metallic native ions, is the probe of the early works in Biology and the most common one even nowadays. ^{111}In has been used to study lipid vesicles, proteins and even DNA. ^{181}Hf appears to be in the third place (8%). In this case all the studies concern almost the same topic: its biodistribution (it is important to remark that Hf can be considered as a surrogate of plutonium). Publications using ^{181}Hf appear between 1986 and 1993. Isotopes of ^{199m}Hg, ^{111}Ag and ^{99}Mo as probes are present in a few publications, all of them in the field of protein studies.

Most of the PAC studies have been carried out *in vitro* as shown in Fig.3. We include here cell culture studies [14]. About 12 % of the total publications correspond to studies *in vivo*. In these cases a discrimination of the biological systems is made: rats and mice [4], bacteria [4] and plants [15]. The first ones being the most studied mainly in relation to lipid vesicles.

Although the scope of PABS covers several aspects (in-vivo metal uptake and dynamics, protein, peptide and nucleic acid dynamics, protein conformational changes, probe site structure, integrity of liposomes in vivo, etc [4]), we have grouped all the works in three general topics: proteins and peptides, liposomes and DNA. This is shown in Fig. 4. The majority of the works correspond to proteins and peptides and this tendency continues in our days. The research in nucleic acid is scarce but publications appear regularly.

In Fig.5 the PABS publications concerning proteins and peptides are discriminated and the most studied proteins can be distinguished. The studies started with carbonic anhydrase. Numerous works appears in transferrin between 1980 and 1994, all of them using ^{181}Hf. The majority of the recent works include research in blue copper proteins (azurin, laccase, plastocyanin and stellacyanin) and beta-lactamase, which is a key protein concerning antibiotic resistance in bacteria.

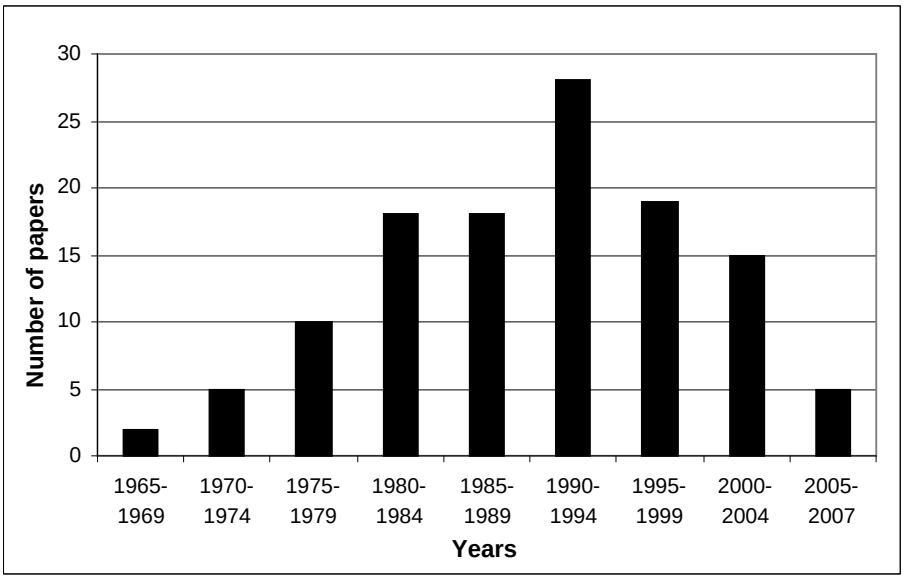

Figure 1. Number of PABS publications versus date of publication.

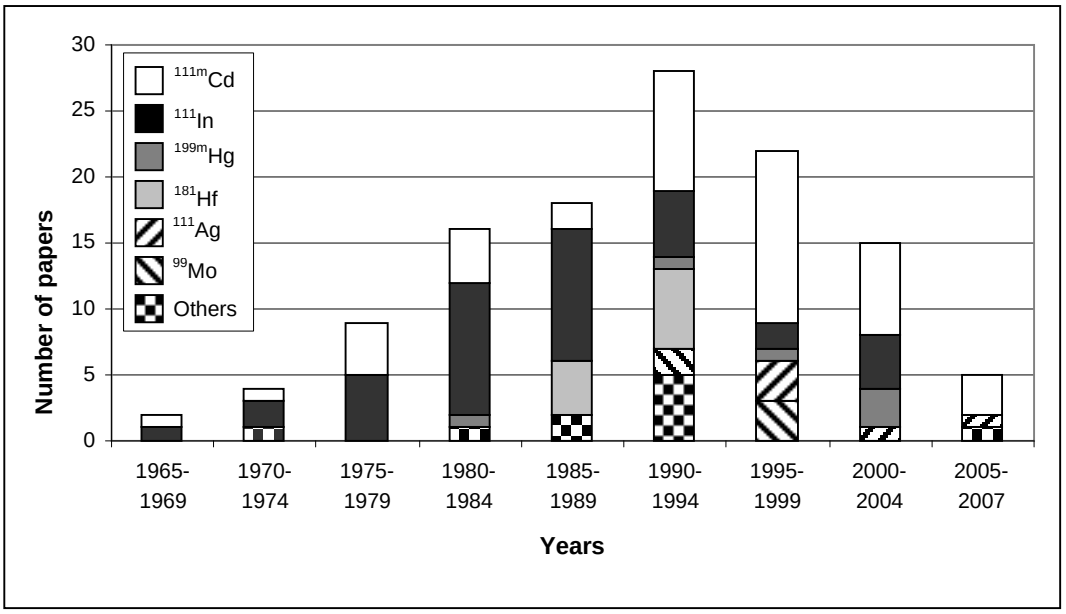

Figure 2. Number of PABS publications versus date of publication indicating the used probes. In "others" are included probes with scarce use (less than 3 papers) as ^{160}Dy, ^{152}Eu, ^{147}Nd, ^{117}Cd, ^{133}Cs, ^{62}Zn, ^{160}Tb and ^{133}Ba.

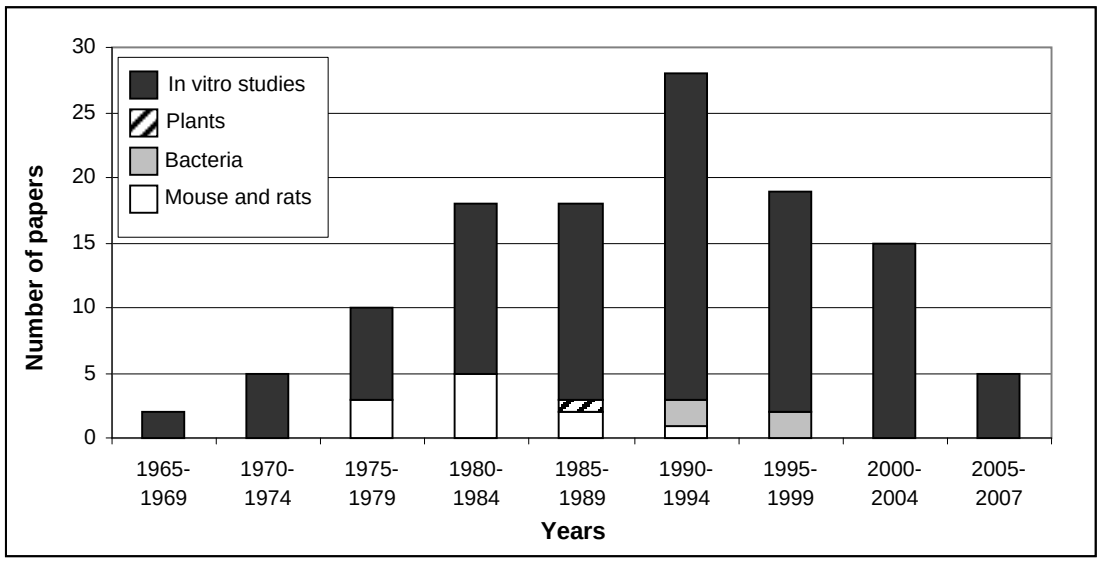

Figure 3. Number of PABS publications versus date of publication differentiating between *in vitro* or *in vivo* studies. In the last group a discrimination of the biological system studied is made.

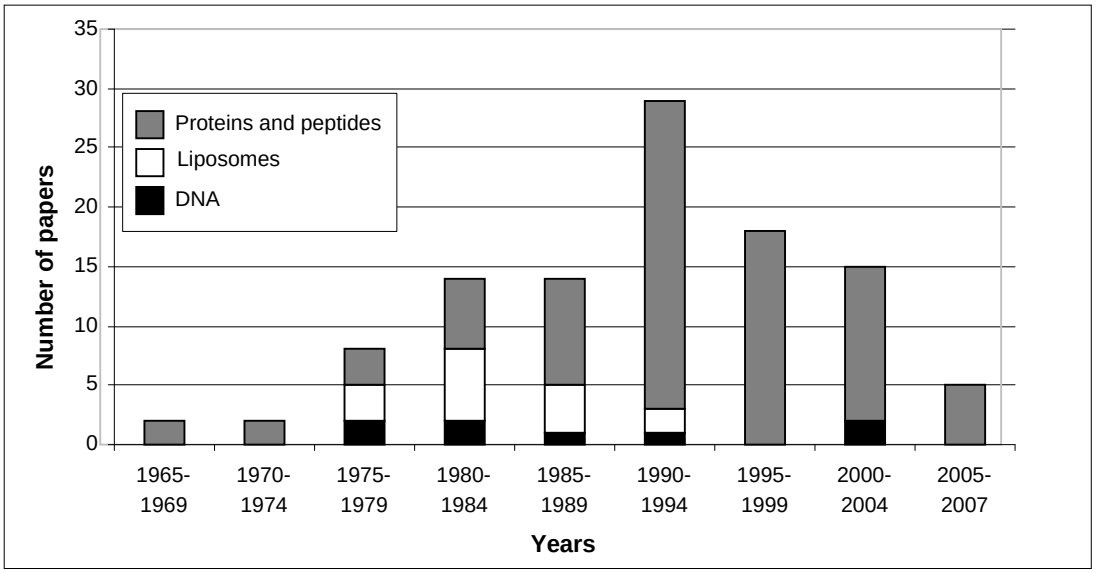

Figure 4. Number of PABS publications versus date of publication indicating the general system investigated

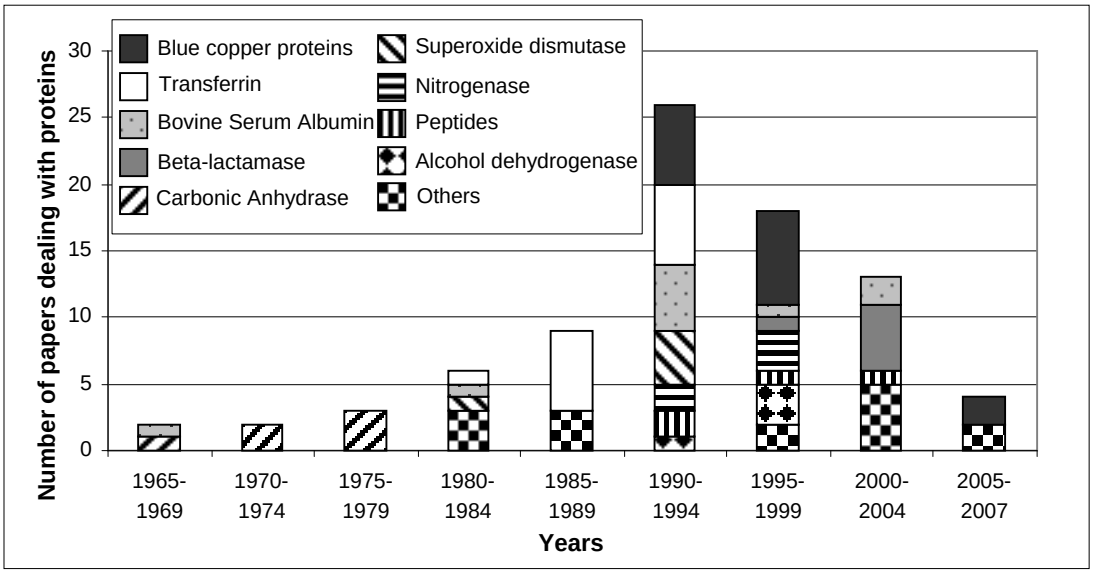

Figure 5. Number of PABS publications dealing with proteins and peptides versus date of publication

3. PAC probes: merits and drawbacks

The PAC technique requires very special isotopes as probes. The best ones are ^{111}Cd and ^{181}Ta. Unfortunately, these isotopes are not common in biological systems neither are their parent isotopes ^{111m}Cd, ^{111}Ag, ^{111}In and ^{181}Hf. Consequently, it is difficult to introduce adequate probes in these systems.

The advantage of ^{111}In over the other PAC isotopes resides in its half period of 2,8 days which enables to perform experiments that require a long time for the sample preparation. Another advantage of this isotope could be that it has several well-established applications in biology other than PABS. The disadvantage concerns the

"aftereffects" of the Electron Capture decay: the Auger electrons produced after the decay leave the probe in a highly ionized state, which in turns destroy the PAC signal. This problem does not exist in the case of the cascade following the disintegration of ^{111}Ag or the desexcitation of ^{111m}Cd.

The ^{111m}Cd isotope can substitute elements such as Cu and Zn that are more common in nature but it has a short half period ($T_{1/2}$=48,6 min) and normally the doping procedure requires isotope separation.

In the case of ^{111}Ag, the $T_{1/2}$ is favourable (7,45 days) but the PAC cascade is weakly populated (5%).

Regarding the ^{181}Hf, it has also an appropiate half-period (42,4 days) and the β-decay, combined with the long lifetime of the initial level of the gamma cascade, does not produce "aftereffects". Unfortunately, this isotope has not important applications in the field of biology.

4. Biological applications using ^{111}In and ^{181}Hf

We report here the applications of some PAC probes other than their use in PAC. ^{111}In can be used in medicine as constituent of radiopharmaceuticals for cancer treatment. The group of K. A. Vallis [16] has developed a radiopharmaceutical called ^{111}In-DTPA-hEGF by combining the radioactive isotope with human epidermal growth factor (hEGF), which targets some tumors. ^{111}In emits densely ionising Auger electrons within the range of nanometers to micrometers and which are highly damaging for DNA. This compound is nowadays under preclinical studies. Lipid-soluble compounds of ^{111}In (for example ^{111}In-8-hydroxyquinoline and ^{111}In-mercaptopyridine-N-oxide), are also under investigation as they passively diffuse through the cell membrane, bind to cytoplasmatic components, and remain bound to the cell until radioactive decay [17].

^{111}In is also used as radiotracer in cardiovascular medicine. The labelled platelets have applications in biomedical investigation and also in clinical diagnosis. The importance of this technique is that many pathological conditions involve the vascular system. In research field, ^{111}In labeled platelets allow to visualize thrombi and distinguish the activation of platelet aggregation in different situations [18].

Additionally ^{111}In labeled peptides and antibodies have been used as chemical and biological surrogates of ^{90}Y therapeutic radiopharmaceuticals (^{90}Y is good for therapies as it is a beta emitter but it is not good for obtaining gamma images). By labeling radiopharmaceuticals with ^{111}In it is possible to study their biodistribution [19; 20]. Recently, ^{111}In has been used in the characterization of innovative pharmaceutical approaches, as it is the case of magnetic targeted carriers [21]. They constitute magnetically susceptible microparticles that can be targeted to specific locations within the body and selectively deliver pharmaceutical agents to cancerous cells, tumors and organs [22].

In all these fields, the PAC technique could contribute to characterize ^{111}In probes environments and to detect the changes that may occur in biological processes.

In turn, hafnium, which is not employed in the manufacture of radiopharmaceuticals, is used in the form of hafnium nitride as a component of the antimicrobial coatings of surgical metal implants [23] and as a non-alpha-particle-emitting analogue of plutonium for *in vivo* studies [24]. Therefore, in the case of hafnium, it seems not possible to make PAC research in fields other than metal transport [3,4] and toxicity of metals [25].

5. Planned PAC experiments

We plan to use ^{111m}Cd produced by the nuclear reaction ^{110}Cd (n,γ) ^{111m}Cd. We will use Cd enriched in ^{110}Cd as target and implant ^{111m}Cd into ice with a mass separator. Afterwards, we will use the water with the activity for doping the samples. We are trying to develop an efficient and quick chemical procedure for doping metalloproteins with Cd and we are making arranges in order to use a PAC lab near enough to a reactor. We will begin with metalloproteinases, which contain Zn, necessary for the enzymatic function (and of easy substitution by Cd) and play an important role in the developing of metastasis [26]. For this reason it is important to understand how to inhibit these proteins and characterize the protein structure with and without inhibiter. Although a lot of work has been made, the old experiments must be repeated as the state of art in PAC has changed a lot from the seventies to now.

As an additional line, we are considering the use of ^{111}In, commercially available as carrier free $InCl_3$. The PAC experiments will be basically oriented to the characterization of probe carriers and radiopharmaceuticals, firstly prepared or commercial and then in the organic tissue where they localize. In this sense, we are preparing PAC experiments on characterization of metalloproteinases and In-DTPA-EGF with ^{111}In. Though aftereffects of the Electron Capture decay could play a negative role working with this isotope, the experiments will have the last word

We also plan to work with the isotope ^{181}Hf as probe. We will try to characterize the hafnium transport in isolated cell systems. As the “in vivo” conditions are difficult to reproduce in culture dish, it is often hard to relate the obtained results with the real life [25], but under appropriate chemical conditions, the metal location could be very well determinate.

Summarizing, we conclude that it is possible to face PABS of interest in the following topics:

- Studies of metalloproteins doped with ^{111m}Cd (revisiting old experiments and making new ones)
- Studies of metalloproteins doped with ^{111}In.
- Studies of platelets doped with ^{111}In.
- Studies of radiopharmaceuticals doped with ^{111}In.
- Studies of Magnetic Targeted Carriers doped with ^{111}In.
- Studies of metal transport in isolated cell systems using ^{181}Hf.

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