BULLETIN N° 181
ACADÉMIE EUROPEENNE INTERDISCIPLINAIRE DES SCIENCES

lundi 6 janvier à 17h Maison de l'AX 5 rue Descartes 75005 Paris

Une interprétation synthétique: "la théorie de la double préparation"
par notre Collègue Michel GONDRAN
Ancien conseiller scientifique EDF/Université Paris-Dauphine
Réflexions sur la finalisation du prochain Colloque :
"Formation des Systèmes stellaires et planétaires Conditions d'apparition de la vie"

Prochaine séance :
lundi 3 février à 17h Maison de l'AX 5 rue Descartes 75005 Paris

CONFÉRENCE
"La résonance magnétique nucléaire, un outil puissant pour la microbiologie"
par Jean-Philippe Grivet
Professeur Émérite à l'Université d'Orléans
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ACADEMIE EUROPEENNE INTERDISCIPLINAIRE DES SCIENCES
FONDATION DE LA MAISON DES SCIENCES DE L'HOMME

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TABLE DES MATIERES

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Fondation de la Maison des Sciences de l'Homme, Paris.

Séance du

Lundi 6 janvier 2014
Maison de l'AX 17h

La séance est ouverte à 17h sous la Présidence de Victor MASTRANGELO et en la présence de nos Collègues Gilbert BELAUBRE, Gilles COHEN-TANNOUDJI, Françoise DUTHEIL, Claude ELBAZ, Jean -Pierre FRANCOISE , Gérard LEVY, Michel GONDRAN, Irène HERPE-LITWIN, Pierre MARCHAIS, Jean SCHMETS, Alain STAHL

Etaient excusés François BEGON, Bruno BLONDEL, Michel CABANAC, Alain CARDON, Alain CORDIER, Daniel COURGEAU, Robert FRANCK, Walter GONZALEZ, Jacques LEVY , Valérie LEFEVRE-SEGUIN , Pierre PESQUIES, Jean VERDETTI.

Etait présent en tant qu'invité, le Pr Jean-Philippe GRIVET de l'Université d'Orléans qui nous est présenté par notre Président en tant notre prochain conférencier du mois de février.

I. Conférence de notre Collègue Michel GONDRAN :

A. Résumé préalable : Une interprétation synthétique: "la théorie de la double préparation" [1]

Au congrès Solvay de 1927, trois interprétations apparemment inconciliables de la fonction d'onde de la mécanique quantique sont proposées: l'interprétation de l'onde pilote par de Broglie, l'interprétation de l'onde soliton par Schrödinger et la règle statistique de Born par Born et Heisenberg. Dans cette présentation nous démontrons la complémentarité de ces interprétations qui correspondent à des systèmes quantiques préparés différemment et nous en déduisons une interprétation synthétique: "la théorie de la double préparation".

Tout d'abord, nous introduisons en mécanique quantique le concept de particule semi-classique préparée statistiquement et nous démontrons que l'équation de Schrödinger de ces particules converge, quand on fait tendre la constante de Planck vers zéro, vers les équations d'un ensemble statistique de particules classiques. Ces particules classiques sont indiscernables et nous concluons à la nécessité de l'interprétation de Broglie-Bohm pour les particules semi-classiques préparées statistiquement (onde statistique).

Puis, nous introduisons en mécanique quantique le concept de particule semi-classique préparée de manière déterministe et nous démontrons que l'équation de Schrödinger de ces particules converge, quand on fait tendre la constante de Planck vers zéro, vers les équations d'une particule classique unique. Cette

[1] Nous vous redonnons ici le résumé déjà présenté dans les documents du bulletin n° 180 car il introduit l'exposé qui suit..

particule classique est discernable et nous concluons à la nécessité de l'interprétation de Schrödinger pour les particules semi-classiques préparées de manière déterministe (onde soliton).

Ces deux théorèmes correspondent au fondement de notre interprétation. Ils s'appuient sur l'analyse Minplus, une nouvelle branche des mathématiques que j'ai developpée à la suite de Maslov, et sur l'intégrale de chemin Minplus qui est l'analogue en mécanique classique de l'intégrale de chemin de Feynman en mécanique quantique.

Enfin, quand l'interprétation semi-classique n'est pas valide, l'interprétation statistique de Born est la seule valide si nous conservons l'équation de Schrödinger. En effet, pour la description des transitions entre les niveaux, la fonction d'onde statistique de Schrödinger ne permet pas, de remonter à un comportement individuel. Nous faisons l'hypothèse qu'il est possible de construire une théorie quantique des champs déterministe (dont nous donnons un début de construction) qui étende au cas non semi-classique la théorie de la double préparation semi-classique précédente.

On peut considérer cette interprétation qui dépend de la préparation de la particule comme une réponse à la "théorie de la double solution" que recherchait Louis de Broglie: "*J'introduisais, sous le nom de « théorie de la double solution » l'idée qu'il fallait distinguer deux solutions distinctes, mais intimement reliées à l'équation des ondes,* l'une que j'appelais l'onde u étant une onde physique réelle et non normable, comportant un accident local définissant la particule et représenté par une singularité, l'autre, l'onde □ de Schrödinger, normable et dépourvue de singularité, qui ne serait qu'une représentation de probabilités" (de Broglie, 1971). Nous avons repris cette idée d'une onde statistique Ψ et d'une onde soliton u, non sous la forme d'une double solution mais d'une double interprétation de la fonction d'onde suivant les conditions initiales de sa préparation.

B. Cela conduit notre Collègue développer l'exposé ci-dessous concernant les points suivants:

- Particules indiscernables en physique statistique
- Particules discernées et indiscernées en mécanique classique
- Convergence de la mécanique quantique vers des particules indiscernées
- Convergence de la mécanique quantique vers des particules discernées
- Nouvelle interprétation de la mécanique quantique

Vous trouverez ci-dessous (p.05 à 12) l'ensemble des diapos afférentes:

Particules indiscernables en physique statistique

Dans son hommage à Einstein lors du centenaire de sa naissance en 1979 [1], Alfred Kastler rappelait que déjà en 1924 " *la distinction entre entités discernables et entités non discernables et la différence de comportement statistique entre ces deux types d'entités étaient restées une chose obscure* ". Et il poursuit : " *Boltzmann avait traité ces "molécules" comme des entités discernables, ce qui l'avait conduit à la statistique dite de Bolzmann. Planck, au contraire, avait traité implicitement les "éléments d'énergie" qu'il avait introduits comme des particules indiscernables, ce qui l'avait conduit à un décompte de probabilité W d'un état macroscopique différent de celui de Botzmann. En 1909, Einstein devait, à juste titre, critiquer ce manque de rigueur.*"

La définition actuelle est toujours aussi floue : "*les particules indiscernables sont des particules identiques qui ne peuvent être différenciées l'une de l'autre, même en principe. Ce concept prend tout son sens en mécanique quantique, où les particules n'ont pas de trajectoire bien définie qui permettrait de les distinguer l'une de l'autre.*" [2] Lorsque la définition devient précise comme "*en mécanique classique, deux particules dans un système sont toujours discernables*" et " *en mécanique quantique, deux particules identiques sont indiscernables* " [3] p.328-329, elle entraîne des paradoxes, comme le paradoxe de Gibbs.

Particules discernées et indiscernées en mécanique classique

Definition

Une particule classique est dite **discernée** si on connait, à l'instant initial, sa position initiale $\mathbf{x}_o$ et sa vitesse initiale $\mathbf{v}_0$.

On ne connait que la densité de probabilité $\rho_0(\mathbf{x})$ et le champ de vitesse $\mathbf{v_0}(\mathbf{x})$ par l'intermédiaire de l'action initiale ($\mathbf{v}_0(\mathbf{x}) = \frac{\vec{\nabla} S_0(\mathbf{x})}{m}$).

Definition

Une particule classique est dite **indiscernée** si on ne connait, à l'instant initial, que la densité $\rho_0(\mathbf{x})$ de sa position initiale ainsi que l'action initiale $S_0(\mathbf{x})$.

N particules identiques préparées de la même façon avec chacune la même densité initiale $\rho_0(\mathbf{x})$ et la même action initiale $S_0(\mathbf{x})$, soumises au même champ de potentiel $V(x)$ et qui vont avoir des comportements indépendants.

N particules indiscernées en mécanique classique

Ces particules indiscernées auront dans les dénombrements les mêmes propriétés que l'on accorde habituellement aux particules indiscernables. Ainsi, si on tire au hasard dans la densité initiale $\rho_0(\mathbf{x})$ N particules identiques, les N ! permutations de ces N particules sont strictement équivalentes et ne correspondent, comme pour les particules indiscernables, qu'à une seule configuration.
On en déduit que, si X est l'espace des cordonnées d'un système d'une particule indiscernée, le vrai espace de configuration d'un système de N particules indiscernées n'est pas le produit cartésien X^N, mais l'espace obtenue par identification des points de X^N représentant la même configuration, c'est-à-dire l'espace X^N/S_N quotient de X^N par le groupe symétrique S_N.

Equations statistiques d'H-J et paradoxe de Gibbs

Théorème

La densité $\rho(\mathbf{x},t)$ et l'action $S(\mathbf{x},t)$ de particules classiques indiscernées vérifient ***les équations statistiques d'Hamilton-Jacobi*** *:*

$$\frac{\partial S(\boldsymbol{x},t)}{\partial t}+\frac{1}{2m}(\nabla S(\boldsymbol{x},t))^2+V(\boldsymbol{x})=0 \qquad \forall\,(\boldsymbol{x},t)\in\mathbb{R}^3\times\mathbb{R}^+ \quad (1)$$

$$S(\boldsymbol{x},0)=S_0(\boldsymbol{x}) \qquad \forall\ \boldsymbol{x}\in\mathbb{R}^3. \quad (2)$$

$$\frac{\partial\rho(\boldsymbol{x},t)}{\partial t}+div\left(\rho(\boldsymbol{x},t)\frac{\nabla S(\boldsymbol{x},t)}{m}\right)=0 \qquad \forall\,(\boldsymbol{x},t)\in\mathbb{R}^3\times\mathbb{R}^+ \quad (3)$$

$$\rho(\mathbf{x},0)=\rho_0(\mathbf{x}) \qquad \forall\mathbf{x}\in\mathbb{R}^3. \quad (4)$$

La vitesse en chaque point (x,t) est donnée par $\mathbf{v}(\mathbf{x},t)=\frac{\nabla S(\mathbf{x},t)}{m}$.

Equations statistiques d'H-J et paradoxe de Gibbs 2

Le paradoxe de Gibbs permet d'étayer la définition des particules indiscernées en mécanique classique. En effet, *lorsqu'on calcule l'entropie d'un mélange de deux gaz identiques, le calcul par la mécanique classique avec des particules discernables conduit à une entropie double de celle que l'on attend. Si l'on remplace ces particules par des particules indiscernables, alors le facteur lié à l'indiscernabilité conduit au bon résultat.*
Ce paradoxe, dénoncé par Gibbs [7] dés 1889, n'a été résolu qu'avec la mécanique quantique plus de 30 ans plus tard, grâce à l'introduction du postulat d'indiscernabilité pour les particules quantiques identiques. Mais, comme le fait remarquer Henri Bacry [6], *"le déroulement historique aurait pu être différent. En effet, en toute logique, on aurait pu postuler le principe d'indiscernabilité pour sauver le paradoxe de Gibbs... Mais ce principe peut être adjoint aussi bien à l'ensemble des postulats de la mécanique quantique qu'à celui de la mécanique classique"*.

Equations statistiques d'H-J et paradoxe de Gibbs 3

Comme le note Greiner, outre le paradoxe de Gibbs, on rencontre de nombreux cas où il faudrait considérer des particules indiscernables en mécanique classique et des particules discernables en mécanique quantique [10] p.151-152 : " *Le facteur correctif de Gibbs est par conséquent effectivement la bonne recette pour éviter le paradoxe de Gibbs. Dorénavant nous tiendrons toujours compte du facteur correctif de Gibbs lorsque nous compterons les états microscopiques pour des états indiscernables. Soulignons cependant que ce facteur n'est qu'une recette pour éviter les contradictions de la mécanique statistique classique. Dans le cas d'objets discernables (par exemple, des atomes qui se trouvent en certains points d'une maille) le facteur de Gibbs ne doit pas être ajouté. Dans la théorie classique les particules restent discernables. Nous rencontrerons encore fréquemment cette inconsistance en mécanique classique.*"

Particules discernées et équations ponctuelles d'H-J

Peut-on définir une action pour une particule discernée ?

Definition

On définit conjoitement **l'action ponctuelle** $S^{\mathbf{x}_0}(\mathbf{x},t)$ et la trajectoire $\xi^{\mathbf{x}_0}(t)$ par les équations d'évolution suivantes

$$\frac{\partial S^{\mathbf{x}_0}(\mathbf{x},t)}{\partial t}|_{\mathbf{x}=\xi^{\mathbf{x}_0}(t)}+\frac{1}{2m}(\nabla S^{\mathbf{x}_0}(\mathbf{x},t))^2|_{\mathbf{x}=\xi^{\mathbf{x}_0}(t)}+V(\mathbf{x})|_{\mathbf{x}=\xi^{\mathbf{x}_0}(t)}=0 \tag{5}$$

$$\frac{d\xi^{\mathbf{x}_0}(t)}{dt}=\frac{\nabla S^{\mathbf{x}_0}(\xi^{\mathbf{x}_0}(t),t)}{m} \qquad \forall t\in\mathbb{R}^+ \tag{6}$$

$$S^{\mathbf{x}_0}(\mathbf{x},0)=m\mathbf{v_0}\mathbf{x} \quad et \quad \xi^{\mathbf{x}_0}(0)=\mathbf{x}_0, \tag{7}$$

que l'on appelera **les équations ponctuelles d'Hamilton-Jacobi.**

L'introduction d'une telle action liée à une trajectoire parait bien étrange et ne présente aucun intérêt opérationnel. Son intérêt est théorique.

Cette action prend son véritable sens lorsque l'on montre qu'elle correspond à la convergence d'un état cohérent lorsque l'on fait tendre la constante de Planck $\hbar$ vers 0.

Théorème

Si $\xi^{\mathbf{x}_0}(t)$ est la trajectoire classique dans le champ $V(\mathbf{x})$ d'une particule de position initiale $\mathbf{x}_0$ et de vitesse initiale $\mathbf{v}_0$, alors il existe une action ponctuelle

$$S^{\mathbf{x}_0}(\mathbf{x},t)=m\mathbf{v}(t)\cdot\mathbf{x}+g(t) \tag{8}$$

avec $\mathbf{v}(t)=\frac{d\xi^{\mathbf{x}_0}(t)}{dt}$ et
$\frac{dg(t)}{dt}=-\frac{1}{2}m\mathbf{v}^2(t)-V(\xi^{\mathbf{x}_0}(t))-m\frac{d\mathbf{v}(t)}{dt}\cdot\xi^{\mathbf{x}_0}(t)$, qui vérifie, conjointement à $\xi^{\mathbf{x}_0}(t)$, les équations ponctuelles d'Hamilton-Jacobi (5)(6)(7).

Convergence vers des particules indiscernées.

$$i\hbar\frac{\partial\Psi}{\partial t} = -\frac{\hbar^2}{2m}\triangle\Psi + V(\mathbf{x})\Psi \qquad \forall(\mathbf{x},t)\in\mathbb{R}^3\times\mathbb{R}^+ \tag{9}$$

$$\Psi(\mathbf{x},0) = \Psi_0(\mathbf{x}) \qquad \forall\mathbf{x}\in\mathbb{R}^3. \tag{10}$$

Dans le changement de variable $\Psi(\mathbf{x},t) = \sqrt{\rho^\hbar(\mathbf{x},t)}\exp(i\frac{S^\hbar(\mathbf{x},t)}{\hbar})$, la densité $\rho^\hbar(\mathbf{x},t)$ et l'action $S^\hbar(\mathbf{x},t)$ sont des fonctions qui dépendent à priori de $\hbar$. L'équation de Schrödinger se décompose en donnant les équations de Madelung [38] (1926) :

$$\frac{\partial S^\hbar(\mathbf{x},t)}{\partial t}+\frac{1}{2m}(\nabla S^\hbar(\mathbf{x},t))^2+V(\mathbf{x})-\frac{\hbar^2}{2m}\frac{\triangle\sqrt{\rho^\hbar(\mathbf{x},t)}}{\sqrt{\rho^\hbar(\mathbf{x},t)}} = 0 \qquad \forall(\mathbf{x},t)\in\mathbb{R} \tag{11}$$

$$\frac{\partial\rho^\hbar(\mathbf{x},t)}{\partial t} + div(\rho^\hbar(\mathbf{x},t)\frac{\nabla S^\hbar(\mathbf{x},t)}{m}) = 0 \qquad \forall(\mathbf{x},t)\in\mathbb{R}^3\times\mathbb{R}^+ \tag{12}$$

avec comme conditions initiales

$$\rho^\hbar(\mathbf{x},0) = \rho_0^\hbar(\mathbf{x}) \qquad et \qquad S^\hbar(\mathbf{x},0) = S_0^\hbar(\mathbf{x}) \qquad \forall\mathbf{x}\in\mathbb{R}^3. \tag{13}$$

Convergence vers les équations statistiques d'Hamilton-Jacobi

$$\rho_0^\hbar(\mathbf{x}) = \rho_0(\mathbf{x}) = (2\pi\sigma_0^2)^{-\frac{3}{2}}e^{-\frac{(\mathbf{x}-\xi_0)^2}{2\sigma_0^2}} \qquad et \qquad S_0^\hbar(\mathbf{x}) = S_0(\mathbf{x}) = m\mathbf{v}_0.\mathbf{x}. \tag{14}$$

avec $V(\mathbf{x}) = -\mathbf{K}.\mathbf{x}$. $\rho^\hbar(\mathbf{x},t)$ et $S^\hbar(\mathbf{x},t)$ sont alors égales à [13] :

$$\rho^\hbar(\mathbf{x},t) = (2\pi\sigma_\hbar^2(t))^{-\frac{3}{2}}e^{-\frac{\left(\mathbf{x}-\xi_0-\mathbf{v}_0t-\mathbf{K}\frac{t^2}{2m}\right)^2}{2\sigma_\hbar^2(t)}} \tag{15}$$

$$S^\hbar(\mathbf{x},t) = -\frac{3\hbar}{2}tg^{-1}(\hbar t/2m\sigma_0^2)-\frac{1}{2}m\mathbf{v}_0^2t+m\mathbf{v}_0.\mathbf{x}+\mathbf{K}.\mathbf{x}t-\frac{1}{2}\mathbf{K}.\mathbf{v}_0t^2-\frac{\mathbf{K}^2t^3}{6m}- \tag{16}$$

$$\sigma_\hbar(t) = \sigma_0\left(1+\left(\hbar t/2m\sigma_0^2\right)^2\right)^{\frac{1}{2}}. \tag{17}$$

σ_0, $\mathbf{v}_0$, ξ_0 et $\mathbf{K}$ sont des constantes données indépendantes de $\hbar$; σ_0 par exemple peut correspondre à la taille du trou permettant de

Quand $\hbar$ tend vers 0, $\sigma_\hbar(t)$ converge vers σ_0 :

Théorème

Quand $\hbar$ tend vers 0, $\rho^\hbar(x,t)$ et $S^\hbar(x,t)$ convergent uniformément vers

$$\rho(x,t) = (2\pi\sigma_0^2)^{-\frac{3}{2}} e^{-\frac{\left(x-\xi_0-v_0t-K\frac{t^2}{2m}\right)^2}{2\sigma_0^2}} \qquad et \qquad S(x,t) = -\frac{1}{2}mv_0^2t+mv_0 \tag{18}$$

solutions des équations statistiques d'Hamilton-Jacobi (1)(2)(3)(4).

Ainsi pour une particule préparée suivant *le cas indiscernée semi-classique*, la densité de probabilité $\rho^\hbar(\mathbf{x},t)$ de la fonction d'onde tend, quand $\hbar$ tend vers 0, vers la densité de probabilité $\rho(\mathbf{x},t)$ d'un ensemble statistique de particules classiques. Nous conjecturons que ce résultat démontré ici pour la particule dans un champ linéaire est général au *cas indiscernée semi-classique*.

CONJECTURE 1 - *Dans le cas indiscernée semi-classique, quand $\hbar$ tend vers 0, pour tout $\mathbf{x}$ et t bornés, la densité $\rho^\hbar(\mathbf{x},t)$ et l'action $S^\hbar(\mathbf{x},t)$, solutions des équations de Madelung*(11)(12)(13), *convergent vers $\rho(\mathbf{x},t)$ et $S(\mathbf{x},t)$, solutions des équations statistiques d'Hamilton-Jacobi* (1)(2)(3)(4).
Cette conjecture se démontre par un calcul explicite pour l'expérience de Stern et Gerlach [19] et pour les expériences,EPR-B [20] par simulation pour l'expérience des fentes de Young. [21]

Interprétation de Broglie-Bohm et courant de spin

Dans *le cas indiscernée semi-classique*, l'indétermination sur la position d'une particule quantique correspond à l'indétermination sur la position d'une particule classique dont on a défini seulement la densité de distribution initiale. **En mécanique classique, on lève l'indétermination en donnant la position initiale de la particule. Il serait illogique de ne pas agir de même en mécanique quantique.**
Nous faisons donc l'hypothèse que, dans *le cas indiscernée semi-classique*, la fonction de Schrödinger représente un ensemble de particules. Une particule quantique n'est donc pas dans ce cas complétement décrite par sa fonction d'onde ; il faut ajouter sa position initiale et il est donc naturel d'introduire les trajectoires de Broglie-Bohm. Sa vitesse à l'instant t est donnée par [37, 28, 33] :

$$\mathbf{v}^\hbar(\mathbf{x},t) = \frac{1}{m}\nabla S^\hbar(\mathbf{x},t) + \frac{\hbar}{2m}\nabla \ln \rho^\hbar(\mathbf{x},t) \times \mathbf{k}, \tag{19}$$

où $\mathbf{k}$ est le vecteur unité parallèlle au spin de la particule.

Convergence des trajectoires de Broglie-Bohm vers les trajectoires classiques

Pour la particule dans un champ linéaire on a :

Théorème

Quand $\hbar$ tend vers 0, la "vitesse quantique"
$\boldsymbol{v}^{\hbar}(\boldsymbol{x},t)=\boldsymbol{v}_0+\frac{\boldsymbol{K}t}{m}+\frac{\left(\boldsymbol{x}-\xi_0-\boldsymbol{v}_0t-\boldsymbol{K}\frac{t^2}{2m}\right)\hbar^2t}{4m\sigma_0^2\sigma_{\hbar}^2(t)}-\frac{\hbar}{2m}\frac{(\boldsymbol{x}-\xi_0-\boldsymbol{v}_0t-\boldsymbol{K}\frac{t^2}{2m})}{\sigma_{\hbar}^2(t)}\times\boldsymbol{k}$
converge uniformément vers la vitesse classique $\boldsymbol{v}(\boldsymbol{x},t)=\boldsymbol{v}_0+\frac{\boldsymbol{K}t}{m}$.

CONJECTURE 2 - *Dans le cas indiscerné semi-classique, quand $\hbar$ tend vers 0, la vitesse $\boldsymbol{v}^{\hbar}(\boldsymbol{x},t)$ et les trajectoires $\xi_{\hbar}^{x_0}(t)$ convergent vers la vitesse classique $\boldsymbol{v}(\boldsymbol{x},t)$ et les trajectoires classiques $\xi^{x_0}(t)$ obtenues à partir des équations statistiques d'Hamilton-Jacobi (1)(2)(3)(4).*
Nous montrons qu'il en est bien ainsi par simulation pour l'expérience des fentes de Young, [21] et par un calcul explicite pour les expériences de Stern et Gerlach [19] et les expériences

Convergence vers des particules discernées quand $\hbar \longrightarrow 0$.

Definition

On dit qu'un système quantique est dans le **cas discernée semi-classique** s'il vérifie les deux conditions suivantes :
- sa densité de probabilité initiale $\rho_0^{\hbar}(\mathbf{x})$ et son action initiale $S_0^{\hbar}(\mathbf{x})$ convergent, quand $\hbar$ tend vers 0, respectivement vers une distribution de Dirac et une fonction régulière $S_0(\mathbf{x})$ indépendante de $\hbar$. C'est en particulier le cas si son action initiale est une fonction régulière $S_0(\mathbf{x})$ indépendante de $\hbar$.
- le potentiel $V(\mathbf{x})$ se conserve lorsque l'on fait tendre $\hbar$ vers 0. Cela suppose que le champ de potentiel peut être décrit classiquement.

C'est le cas lorsque le paquet d'ondes correspond à des états cohérents "quasi-classiques". Ce sont des états introduits dès 1926 par Schrödinger [41] et qui, depuis Glauber (1965). Théoriquement, c'est sur ces états cohérents que sont baties la théorie quantique des champs et la seconde quantification.

Convergence des états cohérents vers les équations ponctuelles d'Hamilton-Jacobi

Dans le cas de l'oscillateur harmonique à deux dimensions, $V(\mathbf{x})=\frac{1}{2}m\omega^2\mathbf{x}^2$, on a :

$$\rho_0^{\hbar}(\mathbf{x})=(2\pi\sigma_{\hbar}^2)^{-1}e^{-\frac{(\mathbf{x}-\mathbf{x}_0)^2}{2\sigma_{\hbar}^2}} \qquad et \qquad S_0(\mathbf{x})=S_0^{\hbar}(\mathbf{x})=m\mathbf{v}_0.\mathbf{x} \tag{20}$$

avec $\sigma_{\hbar}=\sqrt{\frac{\hbar}{2m\omega}}$. Ici, $\mathbf{v}_0$ et $\mathbf{x}_0$ sont encore des vecteurs constants indépendants de $\hbar$, mais $\sigma_{\hbar}$ va tendre vers 0 avec $\hbar$.
Pour cet oscillateur harmonique, $\rho_{\mathbf{x}_0}^{\hbar}(\mathbf{x},t)$ et $S_{\mathbf{x}_0}^{\hbar}(\mathbf{x},t)$ sont égales à [13] :

$$\rho_{\mathbf{x}_0}^{\hbar}(\mathbf{x},t)=\left(2\pi\sigma_{\hbar}^2\right)^{-1}e^{-\frac{(\mathbf{x}-\xi^{\mathbf{x}_0}(t))^2}{2\sigma_{\hbar}^2}} \quad et \quad S_{\mathbf{x}_0}^{\hbar}(\mathbf{x},t)=-g^{\hbar}(t)+m\mathbf{v}(t).\mathbf{x} \tag{21}$$

où $\xi^{\mathbf{x}_0}(t)$ et $\mathbf{v}(t)$ correspondent à la position et à la vitesse d'une particule classique soumise à ce potentiel et ayant $\mathbf{x}_0$ et $\mathbf{v}_0$ comme position et vitesse initiales et où

En notant $g(t) = \int_0^t (\frac{1}{2}m\mathbf{v}^2(s) - \frac{1}{2}m\omega^2(\xi^{\mathbf{x}_0}(s))^2))ds$, on en déduit :

Théorème

Quand $\hbar$ tend vers 0, pour tout $\mathbf{x}$ et t bornés, la densité $\rho^{\hbar}_{\mathbf{x}_0}(\mathbf{x}, t)$ et l'action $S^{\hbar}_{\mathbf{x}_0}(\mathbf{x}, t)$ convergent uniformément vers $\rho_{\mathbf{x}_0}(\mathbf{x}, t) = \delta(\mathbf{x} - \xi^{\mathbf{x}_0}(t))$ et $S^{\mathbf{x}_0}(\mathbf{x}, t) = m\mathbf{v}(t).\mathbf{x} - g(t)$ où $S^{\mathbf{x}_0}(\mathbf{x}, t) = m\mathbf{v}(t).\mathbf{x} - g(t)$ et la trajectoire $\xi^{\mathbf{x}_0}(t)$ sont solutions des équations ponctuelles d'Hamilton-Jacobi (5)(6)(7).

La fonction d'onde converge donc vers le mouvement $\xi^{\mathbf{x}_0}(t)$ d'un oscillateur classique unique. Cette particule classique est complétement définie par sa condition initiale $\xi(0) = \mathbf{x}_0$ et l'action initiale $S_0(\mathbf{x})$. C'est donc une particule discernée.

Interprétations pour le cas discerné semi-classique

L'interprétation de Broglie-Bohm ne s'impose plus mathématiquement. Trois interprétations déterministes et réalistes :
- Dans **l'interprétation de Schrödinger**, la particule quantique dans le cas discerné semi-classique est une particule étendue (soliton), représentée par un paquet d'ondes dont le centre suit une trajectoire classique $\xi(t)$.
- Dans **l'interprétation de Bohr-deBroglie**, la particule quantique est ponctuelle (par rapport à la taille du paquet d'ondes) et suit une trajectoire classique $\xi(t)$ qui est en résonance avec la vibration interne de la particule.
- Dans **l'interprétation de la corde élastique**, la particule quantique n'est pas ponctuelle mais correspond à un modèle spécial de corde élastique dont le centre de gravité suit une trajectoire classique $\xi(t)$. [34, 35]

Enfin, il existe des situations où **l'interprétation de Broglie-Bohm de la fonction d'onde de Schrödinger est surement fausse**. C'est en particulier le cas des transitions entre états propres de l'atome d'hydrogène. **L'approximation semi-classique n'est plus possible** et **il faut utiliser la quantification du champ électromagnétique puisque les échanges se font photon par photon.** Comme le pensait Eistein, on ne peut pas remonter à un comportement individuel déterministe de ces transitions à partir de l'équation de Schrödinger.
Cela ne veut pas dire qu'il faille renoncer au déterminisme et au réalisme, mais que la fonction d'onde statistique de Schrödinger ne permet pas, dans ce cas, de remonter à un comportement individuel.

Nouvelle interprétation de la mécanique quantique

- Dans ***le cas indiscerné semi-classique*** la fonction d'onde ne suffit pas pour représenter les particules quantiques ; il faut lui ajouter leurs positions initiales pour les décrire entièrement et ***l'interprétation de Broglie-Bohm s'impose.***
- Dans ***le cas discerné semi-classique L'interprétation de Broglie-Bohm ne s'impose plus*** car la fonction d'onde suffit pour représenter les particules comme dans l'interprétation de Copenhague. On peut cependant faire l'hypothèse d'une interprétation déterministe et réaliste, complémentaire à celle de Broglie-Bohm, comme celles de Schrödinger, de Bohr-deBroglie ou de la corde élastique.
- Dans les cas où ***l'approximation semi-classique du potentiel n'est plus valable***, ***l'interprétation de Broglie-Bohm de la fonction d'onde de Schrödinger est fausse***. Cela ne veut pas dire qu'il faille renoncer au déterminisme et au réalisme, mais que la fonction d'onde statistique de Schrödinger ne permet pas, dans ce cas, de remonter à un comportement individuel.

II. REFLEXIONS SUR LA FINALISATION DU PROCHAIN COLLOQUE

A. RESUMES DES CONFERENCIERS

Notre Collègue Gilbert BELAUBRE déclare avoir reçu tous les résumés en vue de la rédaction des fascicules fournis aux participants

B. POINT FINANCIER

- Pour limiter les dépenses, les participants pourront prendre des cafés au distributeur
- Un dîner sera offert aux conférenciers ce qui entraînera une dépense comprise entre 800€ et 1200€
- La publication des fascicules fournis aux participants entraînera une dépense d'environ 600€
- Les repas de midi des conférenciers entraîneront une dépense d'environ 21€ par personne
- Le coût des chambres d'hôtel des conférenciers sera de 83€ par personne . 9 conférenciers sont concernés
- Les conférenciers pourront demander donc des prises en charge à leurs universités sur la base des tarifs en vigueur soit ~80€ pour l'hôtel , ~20€ pour les repas . les taxis ne sont pas remboursés, seuls les transports en commun le sont. Les tarifs de notre hôtel et de nos repas sont donc relativement conformes aux tarifs officiels.

C. PUBLICATION DES CONFERENCES

- En vue d'obtenir rapidement les textes complets des conférenciers, notre Collègue Robert FRANCK va leur en adresser très prochainement la demande.

D. DIFFUSION DE L'INFORMATION

- Notre Collègue Gilles COHEN-TANNOUDJI a fait diffuser l'information sur la liste des conférences PIF (Physique et Interrogations Fondamentales)
- Notre Président à fait diffuser l'annonce du colloque sur le site de la SFP (Société Française de Physique)
- Notre collègue Robert FRANCK a fait diffuser l'information sur le site de EDP-Sciences
- Le Secrétariat général a diffusé l'information auprès de :
    - l'AMOPA (Association des Membres des Palmes Académiques)
    - Direction de la communication du CNRS
    - Agence Universitaire de la Francophonie
    - diverses grands organismes universitaires UMPC et organismes dérivés , ENS,
    - des associations telles l'IESF (Ingénieurs et scientifiques de France) la CPGE (Classes Préparatoires aux Grandes Ecoles) , la SFBT (Société Française de Biologie Théorique)
    - Diverses grandes écoles telles l'ENS, ESPCI…….
    - Divers média scientifiques: Pour la Science, Science et avenir, La Recherche..

E. TABLE RONDE

- Christophe MALATERRE ne sera pas disponible, aussi serait-il peut-être souhaitable d'organiser une vidéoconférence avec lui
- Quelqu'un du programme GAÏA serait peut-être intéressant pour la table ronde
- Le journaliste, Sylvestre HUET n'a pas encore répondu. il faudrait peut-être lui trouver un suppléant.

Après cette très riche séance, nos travaux prennent fin.

Irène HERPE-LITWIN

Compte-rendu de la section

Nice-Côte d'Azur

C'est au coeur de l'hiver que j'ai découvert que j'avais en moi un invincible printemps.
Albert Camus.

Compte rendu de la séance du 19 décembre 2013

(176ème séance)

Présents ou représentés :

Jean Aubouin, Richard Beaud, René Blanchet, Pierre Bourgeot, Patrice Crossa-Raynaud, François Cuzin, Guy Darcourt, René Dars, François Demard, Pierre Gouirand, Yves Ignazi, Gérard Iooss, Michel Lazdunski, Jacques Lebraty, Maurice Lethurgez, Claude Nigoul.

Excusés :

Maurice Papo.

1- Approbation du compte rendu de la 175ème séance.

Le compte rendu est approuvé à l'unanimité des présents.

2- Assemblée générale ordinaire.

Comme les compte-rendus - d'activité, moral et. financier - ont déjà été présentés lors de l'Assemblée générale de l'AEIS du 4 novembre 2013 à Paris - Maison de l'AX - et déjà portés dans le bulletin n° 180 de décembre 2013 , nous ne les reproduisons pas.

3 Prochaines réunions et événements:

Prochaine réunion
le jeudi 16 janvier 2014 à 17 heures
au siège : Palais Marie Christine - 20 rue de France
06000 NICE

Prochaine conférence à la Bibliothèque Nucéra
Parking Promenade des Arts
le mercredi 22 janvier 2014 de 17 à 19 heures
Louis Le Sergeant d'Hendecourt
« *Astrochimie et origines de la vie* »
Une approche méthodologique : des comètes à la vie ?
Le futur ne se limite pas à demain ; il a déjà commencé hier.

Annonces

I) Hommage à Emmanuel Nunez, Clinicien, Homme de Science, Systémicien

Conservatoire National des Arts et Métiers, samedi 15 février 2014, salle 21-1-24

Emmanuel Adrien Nunez (1934 - 4 août 2013) vient de nous quitter Il a été Professeur Agrégé de Biochimie enseignant successivement dans les Université de Sousse, St Louis Lariboisière Xavier Bichat. Il a été un grand clinicien, rempli d'humanité, il fut également de 1981 à 2000 le chef de service du laboratoire de Biochimie B du Centre Hospitalier Universitaire Xavier Bichat.

Il a été un Homme de Science. E. Nunez a été directeur de l'unité de recherche INSERM 224 "Biologie comparée des interactions moléculaires entre les protéines, les stéroïdes et les lipides au cours de l'ontogénèse, de l'oncogénèse et de certaines pathologies (choc septique, inflammation, SIDA)" de 1978 à 1994 à la Faculté de Médecine Xavier Bichat. Les recherches de l'Unité 224 INSERM ont été conduites avec des orientations pluridisciplinaires (biochimiques, physiologiques, physico-chimiques, immuno-logiques). Elles ont permis de mettre en évidenceÂ plusieurs propriétés spécifiques de l'Alpha foetoproteine (AFP), proteine carcino embryonnaireÂ : notamment le caractère oestrophile des AFP murines et les hautes affinités de liaison des AFP murines et humaines pour les acides gras non estérifiés essentiels tels que l'acide arachidonique précurseur des prostaglandines et l'acide docosa-hexaénoique abondant dans le système nerveux.

Il a été très impliqué dans l'Académie Européenne Interdisciplinaire des Sciences. Il fut Président de l'Association Française de Science des Systèmes Cybernétiques Cognitifs et Techniques de 2004 à 2009. A ce titre, a présidé la congrès de l'Union Européenne de Systémique de 2005.

Interventions prévues

09h00 - 12h00

- **Marc Fellous (INSERM)**
  in Memoriam
  Emmanuel Nunez et la Tunisie ; sa famille

- **Guy Fitoussi**
  Emmanuel Nunez et la Tunisie ; son enfance

- **Jean-Claude Soufir**
  Emmanuel Nunez et la Tunisie ; son activite à Sousse

- **Gérard Donnadieu (AFSCET)**
  Elements d'une histoire familiale

- **Gilbert Belaubre (AFSCET et AEIS)**
  Hommage à Emanuel Nunez. Autour de son parcours familial

- **Ana Soto et Carlos Sonnenschein (Tufts University)**
  Emmanuel Nunez, une longue conversation sur la science du vivant et la vie bien vécue.

14h00 - 17h00

- **Françoise Ferré**
  Histoire d'une collaboration scientifique

- **Pierre Bricage (AFSCET)**
  Evolution des systèmes vivants : agoantagonisme, rétrogression, émergence. (La biologie des systèmes à partir de l'oeuvre d'Emmanuel Nunez).

- **Vincent Figureau**
  Adaptation du système politique aux changements.

- **Martine Timsit**
  titre à déterminer

- **Jean-Miguel Pire (Ecole Pratique des Hautes Etudes)**
  Art, source de pensée complexe.

II)**ANNONCES CORDIS**

Biologie -Médecine

Update on the advanced neutron tools for soft and bio-materials JRA
Annie Brûlet, the coordinator of this Joint Research Activity, told us about this collaboration's main goals and achievements.
Organisation: NMI3
Country: GERMANY
Category: Press Alert

Event title: 'Plant Gene Discovery & "Omics" Technologies', Vienna, Austria
Date: 2014-02-17
Organiser: For further information, please visit: http://viscea.org/index.php/pgd
Summary: A conference entitled 'Plant Gene Discovery & "Omics" Technologies will be held from 17 to 18 February 2014 in Vienna, Austria. The fast evolving field of gene discovery and in particular 'omics' technologies - indicative of transcriptomics, proteomics, m...
Link to event record: http://cordis.europa.eu/fetch?CALLER=EN_NEWS_EVENT&ACTION=D&CAT=NEWS&RCN=36389

Random chance may explain hereditary disease
A new study from Karolinska Institutet and the Ludwig Institute for Cancer Research shows that random chance decides whether the gene copy you inherit from your mother or the one from your father is used – something which in turn may determine your risk of hereditary illnesses.
Organisation: N/A
Country: SWEDEN
Category: Publication,Result,Press Alert

Nouveaux programmes de recherche

Le montage des projets sous le nouveau programme européen de recherche et d'innovation (Horizon 2020)
Comment rédiger une proposition réellement compétitive? Conseils stratégiques et méthodologiques. Formations dispensées par d'anciens "Project Officers" de la Commission européenne ayant une expérience pratique des projets européens de R&D.
Organisation: Interface Europe
Country: BELGIUM
Category: Event
Horizon 2020: Comment comprendre le nouveau programme européen de recherche et d'innovation
Formations dispensées par d'anciens "Project Officers" de la Commission européenne ayant une expérience pratique des projets européens de R&D
Organisation: Interface Europe
Country: BELGIUM
Category: Calls and Tenders,Event,General Policy,Innovation Service,Legislation,Miscellaneous,Preparatory act,Programme Implementation,Project,Publication,Result,Success Story,Press Meeting,Press Pack,Press Alert,Partnership Event

III) SITE CANALC2 Université de Strasbourg

Depuis 2001, la webtélévision CanalC2 assure la transmission des événements liés à la recherche, à l'innovation et à la culture scientifique, organisés sur toute la France.
Produite par la Direction des Usages du Numérique de l'Université de Strasbourg,
elle permet d'accéder gratuitement à plus de 4700 heures de colloques et conférences filmés, en direct ou en différé.

Je me tiens à votre disposition pour tout complément d'information technique et financier sur nos services de captation audiovisuelle.
Sincères salutations.
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Audrey Bordonné
Assistante de production
Tél : +33 (0)3 68 85 50 13
Fax : +33 (0)3 68 85 00 13
audrey.bordonne@unistra.fr

UNIVERSITÉ DE STRASBOURG

Université de Strasbourg
Direction des Usages du Numérique (DUN)
Département Télévision
Bât. ATRIUM, 3ème Etage
16 rue René Descartes

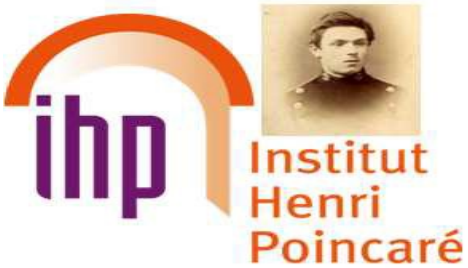
F - 67000 STRASBOURG CS 90032
http://www.canalc2.tv
http://utv.unistra.fr/

IV) RAPPEL concernant notre prochain Colloque des 5 et 6 février 2014:

site inscriptions : **http://aeis-2014.sciencesconf.org**

FORMATION DES SYSTÈMES STELLAIRES ET PLANÉTAIRES CONDITIONS D'APPARITION DE LA VIE

COLLOQUE DE L'ACADÉMIE EUROPÉENNE INTERDISCIPLINAIRE DES SCIENCES



5 et 6 février 2014

A l'Institut Henri Poincaré

Amphithéâtre Hermite

11, rue Pierre et Marie Curie

75005PARIS

L'étude de nos origines est reconnue comme l'un des grands défis scientifiques du XXIe siècle, celle-ci mobilise plusieurs champs conceptuels et combine de nombreux aspects de la connaissance scientifique dans une variété de domaines. Quelle succession d'événements a conduit à la formation des planètes ? Quels processus ont permis l'apparition de la vie sur notre Terre ? Existe-t-il d'autres systèmes sur lesquels une forme de vie pourrait se développer ? Depuis la découverte en 1995 de la première planète extrasolaire de nombreuses autres exoplanètes ont été trouvées dans des configurations qui soulèvent de nouvelles questions sur la formation des systèmes planétaires. De plus, les missions spatiales de ces dernières années ont permis de mieux comprendre la formation du système solaire. Alors que la vie sur Terre se révèle d'une extraordinaire diversité, les progrès récents en astrobiologie permettent d'envisager la recherche d'indices de la présence de vie sur des exoplanètes comparables à la Terre d'ici une ou deux décennies. Ces nouveaux éléments font de la recherche sur nos origines l'un des thèmes majeurs de ce siècle.

Ce colloque a pour objet de présenter les derniers résultats sur les différents aspects de cette recherche interdisciplinaire qui est conduite au plan national, européen et international. La question des origines est si ancienne et profonde qu'elle est aussi développée dans une perspective historique, épistémologique et philosophique. Une table ronde est consacrée à ces aspects.

Comité scientifique

Pierre ALBAREDE (ENS-Lyon), Gilbert BELAUBRE (AEIS), André BRACK (CNRS-Orléans), Sylvie DERENNE (CNRS/ENS Paris), Thérèse ENCRENAZ (IAP), Maryvonne GERIN (CNRS/ENS Paris), Louis LE SERGEANT d'HENDECOURT (IAS- Orsay), Marie-Christine MAUREL (Université PMC-Paris), Pierre NABET(AEIS), Marc OLLIVIER (IAS-Orsay), François RAULIN (Université Paris-Est Créteil), François ROBERT (MNHN), Alain STAHL (AEIS)

Comité d'organisation

Irène HERPE-LITWIN, Gilbert BELAUBRE, Gilles COHEN-TANNOUDJI, Alain CORDIER, Claude ELBAZ, Robert FRANCK, Jean-Pierre FRANCOISE, Michel GONDRAN, Victor MASTRANGELO, Jean SCHMETS, Jean VERDETTI

Contact

irene.herpe@science-inter.com
iherpelitwin@gmail.com

PROGRAMME

Mercredi 5 février /matin

- ***9h00*-9h10** Allocution de bienvenue, Jean-Philippe UZAN, Directeur-adjoint de l'IHP
- **9h10-9h25** Présentation du colloque, Victor MASTRANGELO, Président de l'AEIS

Session 1. Formation des systèmes stellaires et planétaires

Modératrice: Maryvonne GERIN (LERMA/ENS-Paris)

- **9h30-10h05 Nucléosynthèse : L'origine des éléments chimiques dans l'univers**
  Nicolas PRANTZOS (IAP)

10h05-10h15 Échanges avec l'assistance

- **10h20-10h55 La formation des étoiles dans l'univers**
  Patrick HENNEBELLE (LERMA/ENS-Paris)

10h55-11h05 Échanges
11h05-11h15 Pause

- **11h20-11h55 L'étonnante diversité des systèmes planétaires**
  Alessandro MORBIDELLI (CNRS/Observatoire de Nice)

11h55-12h05 Échanges

- **12h10-12h45 Galactic planetary science**
  Giovanna TINETTI (Department of Physics & Astronomy/University College Londres)

11h45-12h55 Échanges

12h55-14h20 Pause déjeuner

Mercredi 5 février/ après-midi

Session 2. Les briques de la vie primitive et les environnements planétaires

Modérateur: François ROBERT (LMCM/MNHN-Paris)

- **14h25-15h De l'Astrochimie à l'Astrobiologie : une approche méthodologique?**
  Louis LE SERGEANT D'HENDECOURT (IAS-Orsay)

15h-15h10 Échanges

- **15h15-15h50 La matière organique insoluble dans les météorites carbonées**
  Sylvie DERENNE (BioEMCo/ENS/Université PMC-Paris)

15h50-16h Échanges
16h-16h10 Pause

- **16h15-16h50 The chemistry of life in terrestrial and non-terrestrial materials**

Raffaele SALADINO (Ecology and Biology Science Department, University of Tuscia-Vterbo, Italy)

16h50-17h Échanges

- **17h05-17h40 Origine de l'eau dans le Système Terre-Lune**
  Francis ALBARÈDE (LGL/ENS-Lyon)

17h40-17h50 Échanges

- **17h55 Fin de sessions**

Jeudi 6 février/matin

Session 3. Transition vers la vie primitive et diversité

Modératrice: Marie-Christine MAUREL (ANbioPhy/Université PMC-Paris)

- **9h-9h35 Premières traces et diversification de la vie**
  Emmanuelle JAVAUX (UPPM/Université de Liège)

9h35-9h45 Échanges

- **9h50-10h25 Eau et sel : premières molécules de la vie**
  Giuseppe ZACCAI (ILL/IBS-Grenoble)

10h25-10h35 Échanges
10h35-10h45 Pause

- **10h50-11h25 L'adaptation microbienne aux environnements extrêmes**
  Bruno FRANZETTI (ELMA/IBS-Grenoble)

11h25-10h35 Échanges

- **11h40- 12h15 À propos de génération moléculaire spontanée**
  Ernesto DI MAURO (Dipartimento BBCD – La Sapienza - Université de Rome)

12h15-12h25 Échanges

12h25-13h55 Pause déjeuner

Jeudi 6 février/après-midi

Session 4. Signatures extraterrestres et modélisation

Modérateur: **Marc OLLIVIER** (IAS-Orsay)

- **14h-14h35 Du système solaire aux systèmes planétaires**
  Thérèse ENCRENAZ (LESIA/Observatoire de Paris-Meudon)

14h35-14h45 Échanges

- **14h50-15h25 Modélisations chimiques du milieu interstellaire**
  Valentine WAKELAM (CNRS/Laboratoire d'Astrophysique-Bordeaux)

15h25-15h35 Échanges
15h35-15h45 Pause

- **15h50-16h25 Morphogenèse et embryogenèse**
  Martine BEN AMAR (LPS/ENS-Paris)

16h25-16h35 Échanges

- **16h45-18h15 Table Ronde « de l'inerte au vivant »,** animée par **Sylvestre HUET**, journaliste et la participation par visio-conférence de **Christophe Malaterre**, Département de Philosophie, Université de Montréal
- **18h20-18h35 Allocution de clôture : Pierre JOLIOT**, Institut de Biologie Physico-Chimique, Collège de France et Académie des Sciences

- **18h40 Remerciements et clôture du colloque**

Documents

Pour préparer l'exposé du Pr Jean-Philippe GRIVET

Nous vous proposons donc :

p.24 : un résumé en anglais de son exposé traduit en français.

p. 25 "NMR and Microorganisms" un article de Jean-Philippe GRIVET paru dans "Curr. Issues Mol. Biol. (2001) 3(1): 7-14"

p.33 extrait d'un article publié dans Progress in Nuclear Magnetic Resonance Spectroscopy 54 (2009) pages 1 à 8 les méthodes expérimentales liées à la RMN utilisées en microbiologie (en anglais)

"La résonance magnétique nucléaire, un outil puissant pour la microbiologie"

par Jean-Philippe Grivet*
Centre de Biophysique Moléculaire CNRS, Université d'Orléans, Rue Charles Sadron, F-45071 Orléans Cedex 2, France

traduit de NMR and Microorganisms
Curr. Issues Mol. Biol. (2001) 3(1): 7-14.

Résumé
Cet article est une introduction à l'usage de la spectroscopie RMN dans l'étude de la physiologie et du métabolisme des micro-organismes. Sont passés en revue les paramètres de RMN qui déterminent la sensibilité et la capacité de résolution de la méthode. Il en découle une vue d'ensemble des applications courantes. Les aspects qualitatifs sont décrits en premier; ils comprennent l'identification des composés et leur localisation. Les aspects quantitatifs tels le pH, la concentration et les mesures des flux sont alors examinés tout comme les contraintes expérimentales correspondantes. L'examen se termine en suggérant de possibles futurs progrès instrumentaux destinés à améliorer la sensibilité: des études plus poussées, des investigations microscopiques en imagerie et en spectroscopie.

Introduction

La première référence à l'application de la RMN aux micro-organismes est celle de Eakin et al. sur le Candida utilis de la candidose (1) . Au cours des 27 dernières années, il y a eu de nombreux développements qui ont fait de la RMN un instrument très utile pour étudier la physiologie et le métabolisme des microbes. Cet introduction examinatrice a pour but de fournir au lecteur une orientation dans le domaine. Elle comprend quatre parties. Nous commençons par une liste de quelques propriétés de la RMN considérées comme importantes pour l'exploration métabolique. Ensuite nous décrivons quelques applications de la RMN, premièrement en tant qu'outil qualitatif, puis quantitatif. Nous avons pris la liberté de mentionner les progrès apparus dans les domaines qui s'y rattachent tels l'analyse des fluides biologiques et les études par RMN des cellules ou des organes de mammifères pourvu qu'elles soient considérées comme utiles et adaptables au cas des micro-organismes. Nous terminons en mentionnant divers développements futurs probables ou désirables

Curr. Issues Mol. Biol. (2001) 3(1): 7-14.

NMR and Microorganisms

Jean-Philippe Grivet*

Centre de Biophysique Moléculaire CNRS, Université d'Orléans, Rue Charles Sadron, F-45071 Orléans Cedex 2, France

Abstract

This article is an introduction to the use of NMR for the investigation of microbial physiology and metabolism. NMR parameters which determine the sensitivity and resolving power of the method are reviewed. A broad survey of current applications follows. Qualitative uses are described first; they include compound identification and localisation. Quantitative aspects, such as pH, concentration and flux measurements are then examined, as well as the corresponding experimental constraints. The review ends with suggestions of possible future developments in instrument capabilities aimed at improving sensitivity: higher fields, spectroscopic and imaging microprobes.

Introduction

The first report on the application of NMR to micro-organisms is that of Eakin *et al.* on the yeast, *Candida utilis* (1). During the past 27 years, many developments have taken place, which make *in vivo* NMR a very useful tool in the study of microbial physiology and metabolism. It is the purpose of this introductory review to provide the reader with an orientation to the field. It is comprised of four parts. We first list a few characteristics of the NMR technique which are deemed important for metabolic investigation. We then describe several applications of NMR, first as a qualitative tool then as a quantitative instrument. We have taken the liberty to mention advances which have taken place in related fields, such as biofluid analysis and NMR studies of mammalian cells or whole organs, whenever they were perceived as useful and readily adaptable to the case of micro-organisms. We end by mentioning various probable or desirable future developments.

Some Aspects of NMR of Importance in Microbiology

We assume that the reader has some knowledge of NMR. We will be mainly concerned with small molecules, observed either in homogenous solutions (supernatant or growth medium, cell extracts) or inside living cells.

Sensitivity

The sensitivity of the NMR experiment should probably be the foremost concern of prospective users. It depends on the following factors.

1) *Nature of the nucleus*: Each magnetic nucleus is characterised by a spin quantum number $I > 0$ and a gyromagnetic ratio γ. Several elements which play an important role in biochemistry have "low γ" nuclei (Mg, Ca) and present special problems. A further point is that nuclei with $I \geq 1$ (such as ^{14}N) have a quadrupole moment and display very broad lines when bound in a molecule. Exceptions include alkali, alkaline earth, and chlorine ions, provided they are not bound to a carrier macromolecule.

2) *Operating magnetic field*: The sensitivity depends roughly on the square of the static field. As the price of a spectrometer is also a steep function of the magnetic field, there is an obvious economic limitation here. It is compounded with the requirement of a wide sample access, because magnet prices also increase with sample bore.

3) *Active volume*: Provided the sample has a correct shape and is relatively homogenous, sensitivity is roughly proportional to the volume inside the radio-frequency coil. Thus, at constant concentration, a 20 mm tube should yield a signal about four times as intense as a 10 mm one.

4) *Concentration of resonant nuclei*: This is the main limiting factor but it depends on many variables, such as the effective concentration of the compound of interest and the relative abundance of the isotope being studied. Many elements have an abundant isotope devoid of magnetic moment while the magnetically active isotope is rare. Such is the case, to varying degrees, for C, O, Ca and S, and also for N because the main isotope is very difficult to use in metabolic studies, due to its broad signal. Enriched compounds are expensive and the range of available substances is limited. The case of hydrogen is almost the opposite; here, the intense solvent resonance tends to swamp the informative signals, and special pulse sequences must be used (2).

5) *Line-width*: The physics of NMR shows that the previous parameters determine the area of each signal. Therefore, the broader the signal, the smaller its amplitude and the more likely that it vanishes amidst noise and baseline artefacts. When working with supernatants or cell extracts, the contributions to the line-widths are those found for all solution work: solids suspended in the medium, paramagnetic impurities, viscous solution; they can all be partly or totally removed. In the case of living cells, the line-width is likely to be dominated by the sample's own heterogeneity. Some causes, such as gas bubbles, can be controlled.

6) *Accumulation time*: The signal to noise ratio increases as the square root of the number of scans accumulated. This result is exact insofar as the sample remains intact (cells remain viable, do not settle, gas bubbles are not formed). Further, the necessary number of scans puts a lower limit on the time resolution of each experiment. It may happen that a decent sensitivity cannot be reached because of limited instrument time and relaxation problems (see next paragraph).

7) *Relaxation delay*: Sensitivity depends on the spin lattice relaxation time T1, the pulse flip angle and the pulse repetition time. A relaxation delay is usually inserted before

*Corresponding author. Email: grivet@cnrs-orleans.fr

each excitation pulse. For quantitative work, a delay of at least $3T_1$ is required if the experimental signal is to be within 10% of the true signal. Shorter delays can be used, along with correction factors. A pair of spectra, recorded with respectively short and very long delays, are used to compute correction factors. This procedure relies on the assumption that relevant parameters do not evolve in time. For solution work, relaxation reagents can be added. They serve two useful purposes: decreasing T_1 and suppressing the Overhauser effect (next paragraph).

8) *Decoupling*: The spectra of nuclei other than hydrogen are usually recorded under complete proton decoupling. Since different nuclei may interact differently with surrounding protons, proton irradiation complicates the recording of quantitative spectra: one must resort to gated irradiation wherein the decoupling effect is retained, but the differential Overhauser enhancement is suppressed. Conditions for quantitative recording have been given (3,4). It is worth noting that the radio-frequency field used for decoupling is attenuated by ionic solutions, an effect which becomes important for large sample volumes and high frequencies. The higher the proton resonance frequency, the larger the spectral width required of the decoupler. One may either use higher decoupler power (at the risk of overheating the sample) or sophisticated decoupling schemes (5,6).

Inverse detection, where heteronuclear resonances are detected via proton signals, is making significant inroads in the field of *in vivo* NMR: it promises important gains in sensitivity and somewhat better resolution (7). The theoretical sensitivity advantage of inverse compared to straight forward detection of nucleus X is $(\gamma_H/\gamma_X)^3$. This large factor may not be fully realised because most reverse probes only accept 5 mm sample tubes and because of the deleterious effect of the intense and broad water signal. The method is also ineffective when applied to nuclei weakly coupled to protons, such as carboxylic carbons. Lastly, quantitation is difficult.

Implicitly, we have been considering the sensitivity required to detect a signal. It is usual to assume that a signal to noise ratio of at least 3 is necessary for detection. Better performance is required for quantification by use of line integrals. It has been shown (8) that the precision of any integral is numerically equal to the inverse of the signal to noise ratio; a signal which is ten times the noise will be integrated to a precision of 10%.

We have left aside the complicated problem of NMR visibility. It sometimes happens that a compound, abundant enough to be detected inside cells, is not seen (or seen with low sensitivity) on the experimental spectrum. A common explanation is that these small molecules are bound to large carrier macromolecules, with very slow motions. The corresponding signals are therefore broadened beyond recognition. A discussion is best left to specific cases.

Resolution

In the early applications of NMR to microbial physiology and metabolism, spectral resolution was rarely an issue. The ranges of ^{13}C and ^{15}N chemical shifts is such that few spectral overlaps were observed. Phosphorus spectra are somewhat crowded, for instance in the phosphomonoester region. This favourable state of affairs is changing because more complex systems are being investigated and also because proton NMR, which has a smaller frequency dispersion, is being used more often. In some cases higher operating fields will alleviate the problem, but the almost definitive solution is two- or multi-dimensional NMR , using multiple labelling and one- or multi-dimensional acquisition and processing. An example is provided by the recent investigation of glyphosate metabolism by Hutton *et al.* (9). Proton NMR investigations have also benefited from 2-D (COSY) techniques; see for instance the review by Navon (10) on proton-phosphorus 2D techniques.

Other Instrumental Requirements

Most experiments useful in metabolic NMR can be done using a typical high resolution spectrometer, provided it has multi-nuclear and reverse detection capabilities. Pulsed field gradients are becoming increasingly useful. They are used to shorten pulse sequences but also to weigh NMR signals according to the diffusion coefficients of the relevant molecules. A spin echo sequence is applied in the presence of a pair of pulsed gradients. The final signal intensity is a function of the diffusion coefficient of the compound under study. Thus, diffusion ordered two-dimensional spectroscopy is fast becoming a powerful analytical technique for complex mixtures (11). As already mentioned, a wide bore and ample spectrometer time are definite advantages.

Modern high resolution NMR puts stringent requirements on the stability of the magnetic field, of the spectrometer electronics and of the sample temperature. Because of the broader line-widths and simpler pulse sequences used, metabolic NMR is less demanding. For *in vivo* experiments, it is often possible to dispense with the field/frequency lock.

Solid state NMR and micro-imaging are two other important techniques. They both require wide-bore magnets, specific probes and special electronics.

Qualitative Information Obtained by NMR

We now turn to an overview of possible applications of NMR spectroscopy related to microbiology. We find it convenient to classify those, somewhat arbitrarily, as qualitative or quantitative.

Identification of Compounds

It is often the case that metabolic pathways produce unknown end products and that NMR is called upon to help in identifying these compounds. This will be particularly true for recently discovered organisms, or for some branches of secondary metabolism. As this type of work is mostly done on supernatants or cell extracts, the investigator can bring to bear all the tools of high resolution, solution NMR. Chemical shifts, signal multiplicities and coupling constant are all useful; two-dimensional correlation maps greatly facilitate the process. The wealth of data available and the fact that NMR, contrary to other characterisation methods, is not specific of any one class

of molecules are clearly advantageous. Some time ago, a novel molecule, 2,3-cyclo-pyro-diphosphoglycerate, was discovered on *in vivo* spectra and fully characterised; its role in gluconeogenesis was later established (12). Other metabolites, including osmolytes (13) and UDP amino sugars (14), have since been described. Kalic *et al.* (15) provide an instructive example (drawn from a study of a particular biofluid) of the power and limitations of this approach.

Identification of Metabolic Pathways or Branches in Pathways

Molecules involved in metabolism have a carbon backbone. It should therefore not be surprising to learn that the most powerful method to investigate a pathway consists in labelling carbon atoms in order to unravel their origin and fate. This can be done with high sensitivity with radioactive ^{14}C labelling, but it is very painstaking to obtain site-specific information inside a molecule. In contrast, ^{13}C NMR provides site information quite easily.

Analogous to the investigations reported in the previous paragraph, NMR spectroscopy can be used to identify new pathways or unknown branches. Given the present state of knowledge, discovering an entirely new pathway is an improbable event, but some steps are often clarified or confirmed by NMR. Again, it is mainly in the case of secondary metabolism, leading to complex molecules, that most applications will be found. We will only mention two extreme examples before referring readers to the references and the "Further Reading" section. Lelait and Grivet (16) used the labelling patterns of amino-acids (obtained after isolation and hydrolysis of proteins) to confirm the main metabolic pathways of acetogenic bacteria growing on ^{13}C labelled glucose or carbonate. Hajjaj *et al.* (17) grew the filamentous fungus *Monascus ruber* on $^{13}C_1$, $^{13}C_2$ and $^{13}C_1$-$^{13}C_2$ labelled acetate to delineate the complex biosynthesis of citrinin.

Hydrogen exchange with the solvent is readily characterised by NMR, provided the solvent atoms can be labelled in some way. Incubating a cell suspension in an $^1H_2O/^2H_2O$ mixture with a ^{13}C labelled substrate is a simple method; site-specific deuterium incorporation in the end products is revealed by the characteristic splitting of the carbon signals under deuterium coupling (18).

The previous paragraphs may have wrongly given the reader an impression that only labelling above the background abundance is useful. This is not the case, as the work of Pasternack *et al.* (19) testifies. These authors incubated *Saccharomyces cerevisiae* in the presence of [1-^{13}C]-glycine and formate in order to delineate one-carbon metabolism. NMR analysis of the metabolic products choline and adenine showed isotopic dilution, thus proving that formate can compete with glycine as a carbon donor.

NMR is also useful for investigations of ecosystems or complex bacterial populations. The presence of homoacetogens in the microflora of human feces was proven by Lajoie *et al.* (20) who detected doubly labelled acetate formed from H_2 and $^{13}CO_2$.

Metabolic Expression in Genetically Modified Organisms

As the physiology and metabolism of naturally occurring micro-organisms become better known, genetically modified bacteria receive closer attention. The main question raised in connection with these organisms can be formulated as follows: are the implanted genes fully expressed or how are the desired functions operating? The work of Ansanay *et al.* (21) provides an illustration. These authors inserted the gene coding for the malolactic enzyme (from *Lactococcus lactis*) into the genomes of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Biochemical analyses, ^{13}C labelling (from [1-^{13}C]-glucose) and comparison of the two species showed that *S. cerevisiae* was indeed able to perform the malolactic fermentation, but with low yield due to a very inefficient malate transport system.

Distinction of Inside and Outside of Cell: Number of Cell Compartments

Several NMR parameters have been used to locate solutes either inside or outside of cells. The first method, historically, relies on the pH dependence of the chemical shift, specially that of phosphorylated compounds (*see* "*pH Measurements*"). The appearance of several "inside" inorganic phosphate signals is a proof of the existence of several cellular compartments, each with a distinctive pH. Another approach makes use of a non-toxic, non-permeating shift and/or relaxation reagent. It is also possible to recognise atoms bound to the outside of the cell membrane.

A more recent technique is based on diffusion weighted spectra (22). The "inside" molecules have a rather low effective diffusion coefficient, while the "outside" ones diffuse faster; moreover, they flow with the perifusion medium. It is possible to completely suppress extracellular contributions to the proton spectrum.

The existence of distinct metabolic pools and the assignment of some intermediates to a specific pool can be established by NMR examination of labelling patterns. The availability of mutants in which specific enzymes are missing is advantageous (19).

Channelling

Eukaryotes harbour multi-enzyme complexes (so-called metabolons). A current hypothesis is that a substrate can move from one active site to another without being released in the cytosol. This motion is assumed to be stereospecific and to preclude label scrambling. Such a mechanism has been investigated in yeast (23).

Quantitative Information

Let us now point to some quantitative measurements derived from NMR observations. The number of such applications is continuously growing, so that we can't even hope to compile a complete list.

Cell Volume

The determination of the volume of intracellular water is required for the calculation of any intracellular metabolite concentration. It is somewhat difficult to perform whatever the technique used, although the principle is simple. A non-permeating inert solute is added to the culture medium and a spectroscopic signal is recorded in the absence and in the presence of the cells. The difference is proportional to the excluded volume, occupied by the cells. On the other hand, the total water volume, both in the presence and absence of cells, is determined from the signal intensities of a freely diffusable compound. The difficulty is that one is looking at small variations of large signals. A careful determination of the intracellular volume for a suspension of *Enterococcus faecalis* by proton NMR is reported in (24).

Diffusion Coefficients

It was mentioned earlier (*see* "*Distinction of Inside and Outside Cell: Number of Cell Compartments*") that spin echo NMR, in the presence of pulsed field gradients, was sensitive to the diffusion of solute molecules. A quantitative determination of diffusion coefficients, both inside and outside the cell is possible. A recent application concerns the structure of granules (flocs) and biofilms. In anaerobic fermenters, bacteria often aggregate in granules, which sometimes show a defined concentric arrangement of bacterial species. It is of great interest to determine the flow of substrates and products in and out of these structures. The same is true for biofilms, whether natural or artificial. First results on water mobility on these structures have been presented (25).

Diffusion measurements can also be used for internal cell volume determination. The technique is more involved than the methods described in the previous section, but more reliable. Using a detailed mathematical model for the restricted diffusion of water with exchange between two compartments, Pfeuffer *et al.* (26,27) could determine volume changes in response to hypoosmotic and hyperosmotic conditions.

Behaviour of Intracellular Macromolecules

Since most enzymes operate inside the cell, one would be quite interested in establishing their properties *in situ*, as opposed to properties determined from cellular extracts, homogenous solutions or even crystals. As already mentioned, the possibility of observing well resolved NMR signals from intracellular macromolecules is remote. It has proved possible in some special cases. Some protons signals of haemoglobin are indeed observable in erythrocytes, because the molecule itself is quite concentrated, there is some segmental mobility and the resonances are shifted to an uncongested part of the spectrum by interaction with the paramagnetic iron atom. A approach which is rather powerful and more general is the biosynthetic labelling of the enzyme with a rare nucleus. This can be accomplished in good yield upon insertion of an inducible expression vector for the relevant gene in the microbe's genome. In the presence of the inductor, the enzyme is synthetised and, if a labelled amino-acid is present in the culture medium, it is incorporated. This technique has been very successfully applied to yeast and to mammalian cells (28). 5-fluorotryptophan was incorporated in various enzymes of the glycolytic pathway or of the Krebs cycle. Rotational correlation times and hence micro-viscosity coefficients could be derived from the observed line-widths.

pH Measurements

This staple of *in vivo* NMR uses either intrinsic probes (mainly phosphorylated compounds) or extrinsic reporter molecules. It is important to construct a calibration curve under conditions as similar as possible to those in the intracellular medium. Many synthetic indicators have been evaluated, including phosphonates (29) and fluorinated compounds (30). Fluorine derivatives have the advantages of a vanishing background and high pH sensitivity, but suffer from the drawback that they require a special spectrometer channel.

Concentrations

Non-invasive, real time, continuous determinations of concentrations represent the main uses of metabolic NMR. A few words of caution regarding experimental procedures have been offered in the section entitled "*Sensitivity*".

Relative concentrations are easily measured, but absolute concentrations are another matter; they can only be defined relative to some reference. The choice of a convenient reference merits some attention. The ideal compound gives a single sharp line well removed from any other signal of interest, does not interact with solutes or cells and is easily handled. For *in vivo* work, it is prudent to use an external reference, i.e. a sealed capillary containing the reference solution inside the main sample tube. An interesting substitute has been suggested (31). A dedicated electronic circuit (or even the decoupler) can be set to generate a signal similar to a free induction decay. This is picked up by the receiver coil, processed as a *bona fide* signal and produces a reference line, thus avoiding any contamination of the sample.

It must be pointed out that different concentrations are measured in biological NMR. Simplest to acquire and interpret are solute concentrations in the culture medium (or supernatant). One is mainly interested in substrates and end products, with perhaps a goal of establishing a carbon balance. Next come cell extracts, for which some biochemical questions must be considered: washing of cells and choice of extraction procedure. Disruption of cells (in a French press or by sonication), followed by protein (or nucleic acid) isolation and hydrolysis puts heavier emphasis on biochemical know-how but provides information on anabolic reactions and pathways. Finally, *in vivo* concentrations rely on free intracellular volume determinations.

To be significant, assays must refer to a known number of cells (or definite cell mass). It appears that the most common unit is the gram of dry cells.

Indirect Determination of Concentrations

In contrast to the direct concentration measurements referred to in the previous section, it is also possible to assay NMR invisible compounds through the effect they have on visible solutes. An early example was the determination of intracellular Mg^{++} through the variation of the chemical shift difference between the α and β phosphorus resonances of ATP (32). Many ^{19}F NMR indicators of Mg^{++} and Ca^{++} have been synthesised and evaluated (33). Oxygen concentrations can, within a limited range, be derived from spin-lattice (T_1) relaxation times.

Kinetics of Individual Reactions

In vivo reaction rates can be measured using the technique of magnetisation transfer. Many versions of this experiment are known (34): steady-state saturation, transient saturation, two-dimensional exchange spectroscopy (EXSY). The principle of the method can be briefly stated. Consider a nucleus that can exist in two chemical environments (say A and B) with distinct resonance frequencies (f_A and f_B) and can jump from one to the other (with rates k_A from A and k_B from B). An example is the ^{31}P nucleus within a phosphate group taking part in the reaction:

$$ADP + PCr \leftrightarrow ATP + Cr$$

The magnetisation of the γ phosphate of ATP evolves under two causes: spin-lattice relaxation and exchange with the PCr magnetisation. In the pulsed version we would look at the perturbation of (say) the ATP signal upon inversion of the PCr resonance (brought about by a weak selective pulse). In effect, we would be watching a competition between chemical exchange, which brings into the ATP site an inverted population of nuclei (negative signal) and spin-lattice relaxation which tends to restore a normal signal. The time scale of such an experiment is the relaxation time T_1 so that reactions much faster or much slower than the spin lattice relaxation cannot be investigated in this manner. The whole process is easily modelled by two first-order differential equations, the solutions of which can be fitted to the experimental results, allowing the determination of the rate constants for exchange. A recent example is given in (35) for the case of a genetically engineered yeast, while Roscher *et al.* (36) show how to apply the EXSY method.

Transmembrane Fluxes

The transport kinetics of ions, substrates or products across the cell membrane can be measured by NMR, provided inside and outside contributions can be recognised. Shift reagents can be used to create a difference in resonance frequencies; many such compounds are available for cation NMR, but interpretation of results can be difficult. The actual rate measurement techniques are quite diverse. If transport is slow, recording of successive spectra after injection of an external compound or resuspension of the cells in fresh medium may allow the monitoring of 'internal' and/or 'external' concentrations as functions of time. In the case of intermediate rate constants, the magnetisation transfer methods of the previous section are suitable. Here, the two exchanging sites are geometrically as well as spectroscopically different.

Because of its high sensitivity and ubiquitous occurrence, sodium has been the subject of many investigations. It has proved, however, rather difficult to quantify the NMR visibility of internal sodium, which has been variously reported to be in the range 30-60% (37,38). ^{23}Na is a spin 3/2 nucleus. When the ion moves slowly, or is bound to a partially oriented macromolecular structure (such as the cyto-skeleton), double and triple quantum transitions become observable and provide a possible technique to assay internal sodium. A complete theoretical treatment is available (39).

Potassium is difficult to observe and at least two substitutes have been proposed: rubidium and caesium. $^{133}Cs^{+}$ would seem to offer two advantages (40). Its quadrupole moment is 200 times lower than that of sodium, promising rather sharp lines, and its chemical shift is so highly sensitive to the environment that the internal and external ions have spontaneously resolved resonances.

The transport of organic solutes can be examined in much the same way, but it is usually slower than that of ions. The case of ethanol efflux from *Zymonas mobilis* is an exception. It is fast enough for magnetisation transfer methods to be applied (41).

Metabolic Flux

Metabolic fluxes, or ratio of fluxes at branch points, are the main quantitative data sought by workers interested in metabolism. They are the equivalent of the V_{max} and K_m of enzymology. They cannot be determined from NMR measurements alone when using a single label. This fact was well known of the practitioners of radioactive labelling who coined the term 'specific activity' (counts per minute per gram of product). It is sometimes masked in NMR; because of its low sensitivity, this analytical technique operates not with tracer amounts but with labelled compounds which form a notable proportion of the substrate. The difficulty can be traced to the fact that isotopic equilibrium (or flux) is different from substrate equilibrium (or flux). In order to stress the point, we submit to the reader a somewhat far-fetched analogy. Suppose that we wish to determine the flow rate of a mountain stream. This is rather laborious to do; we would have to measure the (cold) water speed at many points of the stream cross-section and sum all contributions. Instead, we may decide to dump a known number of fishes into the water and count how many of them pass under a bridge downstream. This method is the analogue of the simple NMR approach mentioned above.

The enrichment of a product P, that is the ratio [labelled P]/[total P], must be known before any conclusion on the corresponding flux can be drawn. The amount of P in a culture can be, in principle, determined with any analytical technique. For instance, Dominguez *et al.* (42) combined acidic and basic extraction procedures, HPLC and fluorescence assays with ^{13}C NMR analysis of labelling patterns to determine carbon fluxes in the central metabolism of *Corynebacterium glutamicum*.

Combining results derived from several techniques and several samples should be considered with some caution. Several investigators have proposed methods using only NMR. One approach (43) uses proton NMR and ^{13}C labelling; on the proton spectrum, both unlabelled and labelled product can be observed, provided the C-H coupling constant is large enough, as is the case for CH_n groups. Wendisch *et al.* (44) have developed a frequency selective spin echo difference technique which remains useful for non-protonated carbons. Any magnetically active nucleus coupled to carbon could be used; Lutz *et al.* (45) have taken advantage of ^{31}P-^{13}C couplings to determine the specific enrichment of phosphorylated metabolites.

The most powerful method is probably the analysis of amino acid multiplet patterns arising when micro-organisms are fed with uniformly labelled glucose, as first described by Gagnaire and Taravel (46) and perfected by Szyperski *et al*. One is sometimes interested in the global turnover or renewal rate of a whole family of molecules, for instance amino acids. Roberts *et al*. (47) used ^{15}N NMR to measure the free amino-acid turnover rate in methanogens.

Up to now, we have been mainly concerned with pure species; however, some quantitative work has been done on mixed cultures and complete ecosystems. Wolin *et al.* (48) examined the colonisation of the digestive tracts of infants by Bifidobacteria. These micro-organisms use a unique pathway of hexose catabolism to produce acetate and lactate. One third of the acetate molecules are derived entirely from the C_3 carbon. Fermentation of [3-^{13}C]-glucose thus yields doubly labelled acetate, with a characteristic spectrum. It was shown that 70% of the acetate produced by a bacterial suspension was due to Bifidobacteria.

Are Isotopic Substitutions Innocuous?

After reading the previous sections, the reader has no doubt noticed that isotopic labelling is one of the main tools of metabolic NMR. He or she may well wonder whether substituting a nucleus by a lighter or heavier isotope has any effect on the biochemical reactions being studied. A short answer is no, except possibly for the hydrogen isotopes. Isotope effects on chemical and biochemical reactions have been extensively studied during the last fifty years. Chemical equilibria are slightly displaced upon isotopic substitution, but the effect is too small to have observable consequences on living systems.

Of greater import are the modifications of rate constants. Hydrogen exchanges can be slowed down by a factor of ten when protium is replaced by deuterium. This is probably the reason why eukaryotes and higher organisms cannot live on heavy water. Kinetic isotope effects are much smaller for heavier nuclei. Organic reactions rates can be reduced by a few percent when ^{12}C is replaced by ^{13}C. The resulting small concentration changes can be monitored by mass spectrometry and have been used as probes of reaction mechanisms (49).

Metabolic pathways operating in micro-organisms comprise many enzymatic reactions; a given metabolite is often synthesised by several routes. The end result is that apparent isotope effects become small (a few per mil, not detectable by NMR) and difficult to interpret, except when they can be related to a single rate limiting step. The $^1H/^2H$ couple is again an exception, with variations in relative abundance of several percent. We refer the reader to the literature (50) for the many interesting applications of site-specific natural isotope fractionation.

Probable and/or Desirable Developments

We will end by listing some technical developments that should in the near future make NMR more productive or easier to use for the microbiologist. This list is not speculative, as the technology already exists for these improvements.

High Field, Wide Bore Magnets

Higher fields will mean more sensitivity, allowing observation of low γ nuclei and/or the use of lower enrichment for rare nuclei. As structural biochemists reach for ever increasing fields, one may hope that many 11.7 T (500 MHz for protons) spectrometers will be converted to metabolic work. A similar trend is seen in biofluid analysis, although here the driving force is the necessity of improving the resolution of proton spectra.

Microprobes

Manufacturers as well as several research groups (51) are developing probes for very small volumes (1-100 microlitres). They could be used in conjunction with very small scale cultures.

High Pressure Probes and NMR of Gases

It is now possible to do high resolution NMR under pressures of 500 MPa (5000 atm)(52). It is conceivable that gas consumption and production (H_2, CH_4) by methanogens or acetogens and interspecific hydrogen transfer could be studied *in vivo* with these specialised probes.

Instead of increasing the effective concentration of a gas, one could think of increasing the apparent magnetic moment (or the γ factor) of the nuclei. This seemingly ludicrous goal can be reached in two cases: ^3He and ^{129}Xe, using optical pumping techniques (53). Although of doubtful biochemical significance in themselves, these gases could serve to explore the geometric structure of microbial aggregates (flocs, granules), much as is now being developed for lung and vascular imaging.

Spectroscopic Imaging

Magnetic resonance imaging, or MRI, is a well known non-invasive investigative technique, useful in material science and in medicine. It is usually implemented with large, horizontal bore magnets. A micro-scale version of MRI uses a vertical bore magnet. The ultimate resolution is estimated as 10 micrometers, although current images show details at about 50 micrometers. This is sufficient to investigate heterogeneous samples, such as granules, biofilms or bioreactors as mentionned earlier (*see* "*Diffusion Coefficients*"). Using special pulse sequences, it is possible

to perform spectroscopic imaging, wherein a three-dimensional concentration map of several abundant metabolites is constructed for an organ such as the brain. The spatial resolution is of the order of half a centimetre. The challenge consists in combining a high spatial resolution and chemical sift information.

Data Banks and Data Processing

To this author's knowledge, no NMR data bank useful for metabolism exits at the present time. The many compilations of chemical shifts that do exist mainly concern organic chemistry: spectra were recorded in organic solvents, for neutral molecules. We believe that it would be useful to collect chemical shifts, coupling constants, pK's of molecules frequently encountered in metabolic work, along with an accurate description of conditions: pH, ionic strength, concentration, temperature.

The use of NMR spectroscopy for research in physiology or metabolism involves the recording and analysing of a great number of spectra and there is a need for the automation of the whole process. Software for the automatic treatment and analysis of spectra is available but, in the author's opinion, no single program can yet handle the variety of spectra and practical problems met when performing *in vivo* NMR.

Conclusion

We hope that this overview of possible NMR applications in microbiology will encourage readers to consider the use of NMR whenever a problem arises in their own research.

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NMR for microbiology: *In vivo* and *in situ* applications

Jean-Philippe Grivet [a,*], Anne-Marie Delort [b]

[a] *Université d'Orléans et Centre de Biophysique Moléculaire, CNRS, rue Charles-Sadron, 45071 Orléans cedex 2, France*
[b] *Laboratoire Synthèse et Études de Systèmes à Intérêt Biologique, UMR 6504 CNRS, Université Blaise-Pascal, 63177 Aubière cedex, France*

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* Corresponding author. Fax: +33 238631517.
E-mail addresses: grivet@cnrs-orleans.fr (J.-P. Grivet), A-Marie. DELORT@univ-bpclermont.fr (A.-M. Delort).

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1. Introduction

Microbes form an extremely rich and diverse set of organisms. They are investigated at a fundamental level, because they are the simplest living organisms and because, in contrast to animal or plant cells, a microbe can live independently from other cells. They are ubiquitous in all earth compartments: in the atmosphere, in soils, in surface, and ground waters and their metabolism is involved in all biogeochemical cycles. They can live under very variable conditions and survive to extreme environments. They can thus respond and adapt their metabolism to fast and strong changes. They are also studied from a more applied point of view because of their role in the digestive tract of animals, in many diseases, in the production of foods and beverages, in industrial and agricultural processes, in pollution

remediation, wastewater plant treatments and, in recent years, in biotechnology.

The applications of nuclear magnetic resonance spectroscopy (NMR) and of nuclear magnetic resonance imaging (MRI) in microbiology reflect this enormous diversity of subjects. The area of study is steadily expanding in response to the continuous improvement of NMR techniques, most importantly improvements in sensitivity. Progress in information technology has been all important. It has influenced NMR itself through Fourier transforms, digital filtering, linear prediction or maximum entropy methods. Readily available data banks provide information on chemical shifts and coupling constants, genes, enzymes, and metabolic reaction networks. Computer models are indispensable in the quantification of metabolic fluxes and in the statistical analysis of spectra (metabolomics). It will be seen in the following that advances in other areas, such as molecular biology, genetic engineering, or enzymology, also have a beneficial or synergistic impact on NMR applications in microbiology.

In order to keep this review within manageable proportions, we have excluded the characterization and the study of the properties of many compounds synthesized by microbes, such as antibodies or antibiotics. We have tried to restrict ourselves to phenomena and molecules rather directly related to the metabolism or physiology of microbes, with the exception of the degradation of xenobiotics. We also have chosen to study compounds of interest in "microbiological NMR" in unpurified form. Samples examined by *in vivo* or *ex vivo* (*in situ*) NMR are whole cells, cell extracts, culture media, soil samples, etc., therefore NMR studies of purified proteins, polymers and biotransformation products are not presented in this review. The use of isotopically substituted molecules as tracers is prevalent in this area. Only a few nuclei are readily accessible, *e.g.* ^{2}H, ^{13}C, ^{15}N, ^{19}F, ^{23}Na, and ^{31}P. This is not an important handicap, since the corresponding elements make up most of organic matter. Moreover, knowledge of the fate of the carbon skeleton is usually enough to define a metabolic process. Other, so-called "exotic" nuclei are outside the scope of this review. However, it should be noted that the number of ^{1}H NMR studies of samples at natural abundance is steadily increasing, especially concerning applications in xenobiotic biotransformation and metabolomics.

Our subject has rarely been reviewed in the past [1,2]. This could reflect the fact that microbiologists were loath to use physical techniques except microscopy, or that NMR specialists considered, for some reason, that plants, organs, animals or human beings were more interesting than microbes. In this respect, one should remember that the average human harbours in his digestive tract more bacteria than there are cells in his body. There is an abundance of reviews on many areas of biological magnetic resonance and they can be of interest to microbiologists. Two very recent articles are useful complements to our contribution. The review by Schneider [3] focuses on the secondary metabolism of plants, while that of Fan and Lane [4] is devoted to metabolomic studies of plants, animals, and humans. As these works provide careful surveys of experimental NMR methods, these will not be taken up here, except for specific cases.

2. Experimental methods

NMR spectroscopy has a distinctive characteristic compared to other spectroscopic methods: important technical improvements have emerged steadily since its invention. We consider separately liquid-state and solid-state spectroscopies.

2.1. Liquid-state experiments

With respect to applications of liquid phase NMR in microbiology, advances in two directions are deemed to be particularly important: increases in sensitivity and development of accurate quantitative methods. Even though no specific application has yet emerged, they are therefore briefly reviewed below.

2.1.1. Sensitivity of NMR experiments

We first address sensitivity improvements. The sensitivity of the NMR experiment is usually expressed as the signal to noise ratio (SNR), for which the following expression has been derived [5,6]

$$\mathrm{SNR} \propto \frac{\omega B_1 M v_s}{\sqrt{4k_B[R_c T_c + T_a(R_c + R_s) + R_s T_s]\Delta\nu}} \tag{1}$$

where ω is the Larmor angular frequency, B_1 is the rf field component in the xy plane generated by unit current in the coil, M is the magnetisation of the sample just before the read pulse, v_s is the sample volume, k_B is the Boltzmann constant, R_c is the rf coil resistance, T_c is the rf coil temperature, R_s is the effective sample resistance which can be considered as connected in series with the rf coil and corresponds to the energy dissipated in the sample, T_s is the sample temperature, T_a is the noise temperature of the preamplifier, and $\Delta\nu$ is the receiver bandwidth. In the case of direct detection, with long delays between pulses, M equals the equilibrium magnetisation M_0 given by Curie's law

$$M_0 = N\frac{\hbar^2\gamma^2 I(I+1)}{3k_B T_s}B_0$$

with γ, the gyromagnetic ratio of the observed nucleus, I its spin quantum number, N the number of nuclei per unit volume, and B_0 the static magnetic field.

Efforts to improve the sensitivity of the NMR experiment for liquids have addressed every factor of Eq. (1) except the sample temperature. If the sample is not mass limited, then an obvious target is the sample volume. As there is a trade-off between resolution and volume, large samples are

mainly used in cases where resolution is not at a premium, most notably for *in vivo* work (see Section 2.3).

2.1.2. Cryoprobes

In cryoprobes [7–9], the coil and preamplifier temperatures are lowered to about 30 K, and the coil resistance can also be reduced by resorting to high-temperature superconducting materials [10]. The sample resistance depends on the nature and concentrations of electrolytes present in the solution and guide-lines have been provided to help in choosing the best buffer system [11–13]. Losses in the sample also depend on the geometry of the electric field induced by the rf pulse and the sample tube cross-section may be optimised to keep the sample in a low electric field region [14].

Most recent investigations have concerned mass-limited samples. This may be because the compounds of interest can be obtained only at low concentrations in solution and cannot be easily labelled (as is the case for xenobiotics), or that labelled substrates are too expensive, or because one is interested in assaying fractions from a chromatographic separation. It is then advantageous to use a probe designed for the small amount of material available and also to optimise the other factors in Eq. (1). The filling factor of a probe is defined as the ratio of sample to coil volumes, $f = v_s/v_c$. It should be close to unity. Further, the intensity of B_1 should be maximised. These two goals are much better achieved with solenoid coils (or with a Helmholtz pair) than with the usual saddle-shaped design [15,16] and solenoid microcoils are now commercially available, with volumes of 5 μL (1 mm diameter sample tubes) or 30 μL (1.7 mm diameter, cryoprobe). A triple resonance 1 mm cryoprobe incorporating superconducting coils in a Helmholtz configuration has recently be described [17]. The reported mass sensitivity is about 20 times that of a conventional 5 mm probe. A review of commercial probes is available [18].

2.1.3. Microprobes

Microfabricated spiral surface coils are useful for *in vivo* and imaging applications. They can be combined with microfluidic devices [19,20]. A commercial probe (Bruker) boasts a spatial resolution of 10 μm in each direction (1 pL voxel volume).

Furthermore, several designs have been proposed which bear little likeness to coils [21]: they derive from the stripline [22], see Fig. 1, or microstrip [23], see Fig. 2, concepts. In these probes, the rf electric field is mostly confined in the dielectric, thus limiting sample losses, and the rf magnetic field circles around the stripline. These set-ups can deliver good sensitivity with 10–30 nL samples. They presently suffer from limited homogeneity essentially because they do not include susceptibility matched materials.

2.1.4. Dynamic nuclear polarisation

Manipulating M, the magnetisation, is a well-known method used to increase the sensitivity for low γ and/or

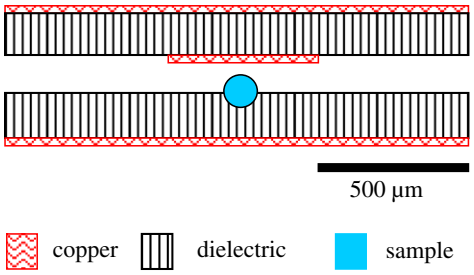

Fig. 1. Outline of the stripline design of van Bentum et al. [22] shown in cross-section.

low abundance nuclei. Proton excitation and detection are now generally accepted for ^{13}C spectroscopy, because the sensitivity of an indirect experiment is proportional to $\gamma_{exc}\ (\gamma_{det})^{3/2}$, where γ_{exc} is the gyromagnetic ratio of the excited nucleus, γ_{det} that of the detected nucleus. Several other nuclear polarisation methods are known and have proved useful in specialized areas: chemically induced nuclear polarisation (CIDNP, [24]), *para*-hydrogen induced polarisation (PHIP, [25]), magnetisation transfer from polarised helium or xenon [26], and electron–nucleus dynamic nuclear polarisation (DNP for short).

This last technique is far from recent; it was proposed by Jeffries [27], Erb et al. [28], and by Abragam and Proctor [29]. Also called the "solid effect", it differs from the Overhauser effect in that it requires a static interaction between electrons and nuclei and that forbidden transitions (at a frequency equal to the sum or difference of the electron and nucleus Larmor frequencies) are driven [30]. For a long time, the main application was the production of polarised

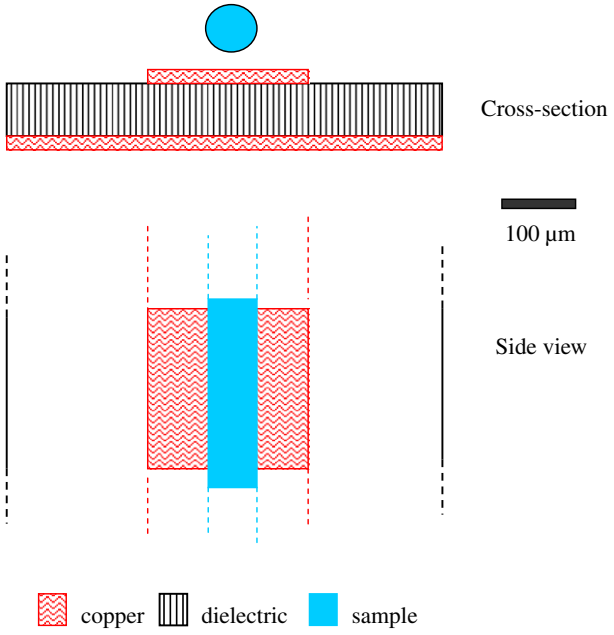

Fig. 2. Outline of the microstrip/microslot probe of Maguire et al. [23] shown in cross-section and side-view. The narrow channels of the microstrip act as inductances; the rf magnetic field lines thread around them.

targets for nuclear physics experiments. However, in a long series of reports, Griffin and co-workers have described the development and application of DNP to the observation of ^{13}C in solid samples under MAS conditions (see Hu [31] and references therein). It was recently realised that, due to the long relaxation times of hetero-nuclei, these could be polarised in the solid-state and the sample then quickly dissolved at room temperature while preserving most of the polarisation [32,33]. It is hoped that this method will be useful in metabolic studies, analogous to the use of short-lived radioactive isotopes in medical imaging.

2.1.5. Intensity calibration

As is well-known, the usual NMR experiment is difficult to make quantitative. This is due in part to the sample itself: nuclei with widely different relaxation times will give different responses. Other problems are inherent to the detection technique: the decoupling efficiency will depend in particular on the frequency offset, artefacts of multi-pulse experiments will make quantitation difficult. Absolute quantitation raises another type of problems: which standard should be used? As these questions have been addressed several times before (for a recent review see Malz and Jancke [34]), we will only mention some recent contributions.

An interesting solution to the quantitation problem was provided by Akoka and co-workers through the ERETIC method [35,36]. It consists in electronically generating a reproducible FID-like signal which is picked up by the probe and processed by the spectrometer, thus providing a secondary standard, without the constraint of adulterating the sample.

The same group has published a series of detailed investigations of the decoupling process in the context of quantitative NMR [37–39]. Highly accurate concentrations may be obtained using well chosen, if somewhat complicated decoupling schemes.

2.1.6. Sampling in the indirect (t_1) direction

Insufficient spectral resolution can be a limiting factor in the analysis of cell extracts, even in the case of crowded two-dimensional spectra. The resolution in the indirect direction can be increased by using more t_1 increments, with a correspondingly longer measurement time. This unfortunate trade-off can be circumvented, as shown in two recent papers. Wagner et al. [40] propose to use randomly chosen t_1 values, (either with a constant average density along the t_1 axis or with an exponentially decreasing density) and a spectral reconstruction algorithm akin to maximum entropy. They are thus able to record ultra-high resolution ^{13}C–^{1}H HSQC spectra in 1/7th the time of the classical method. Jeannerat [41] also aims to reduce the number of increments in the indirect dimension of heteronuclear 2D experiments, although in a completely different approach. When a small spectral width is used, the carbon spectrum is folded or aliased. It proves possible, using a dedicated computer algorithm, to choose the frequency offset and the (strongly reduced) spectral width such that no carbon peaks overlap. The number of time increments is reduced by at least an order of magnitude.

2.2. Solid phase experiments

Magic angle spinning (MAS) was originally proposed by Andrew et al. [42] and by Lowe [43] to overcome the large homonuclear dipole–dipole broadening of ^{1}H spectra of solids. A nice introduction to the theory of MAS can be found in Prof. Schurko's web pages [44]. When a solid sample is spun about an axis making an angle θ with the static magnetic field, the spin Hamiltonian becomes a sum of constant and time-dependent terms. In the case of the dipole–dipole interaction, the time-independent part comprises a $(3\cos^2\theta - 1)$ factor. If $\theta = \theta_m = \arccos(1/\sqrt{3}) = \arctan\sqrt{2}$, the magic angle, this term vanishes. The time-dependent terms are periodic functions of time and cause the FID to be a train of "rotational echoes", separated by a spinner period. After Fourier transformation, a spectrum is obtained which shows spinning side-bands, separated by multiples of the spinning frequency. At low speeds, the spectrum appears quite cluttered but, if the spin rate is larger than the dipolar linewidth, then dipole–dipole interactions effectively disappear. A recent review of the technical requirements of solid-state MAS NMR is available [45].

2.2.1. Rotational echoes

Rotational echoes are put to good use in the REDOR technique proposed and mainly developed by Schaefer and his group [46,47]. In this method, an observed spin S is assumed to be dipolar coupled to an unobserved spin I. As stated above, the S spin FID is a train of echoes. If π pulses are applied to the I spin each half rotor period, the periodicity of the I–S interaction is destroyed and the echo amplitude is diminished: the S spins dephase due to the local field created by the I spins. The difference between an S NMR spectrum obtained under these conditions and one obtained with no I pulses measures the I–S coupling, which scales as r_{IS}^{-3} thus allowing an accurate distance measurement.

2.2.2. High resolution magic angle spinning (HR-MAS)

Schaefer and Stejskal discovered [48] that the chemical shift anisotropy could be averaged by magic angle spinning. It was later realised [49] that all interactions described, as the dipole–dipole part of the Hamiltonian, by a second rank tensor term (heteronuclear dipole–dipole interactions, chemical shift anisotropy, magnetic susceptibility inhomogeneities, first order quadrupole interaction) involve the same angular dependence as the dipole–dipole interaction and would be averaged out. This was the starting point of what came to be known as HR-MAS, *i.e.* magic angle sample spinning within a high-resolution magnet. In principle, this experiment could be performed in a solids probe. However, components of such probes are not susceptibility matched as in their high resolution coun-

terparts and the residual linewidth is too large. HR-MAS has become the preferred NMR method for the study of soft matter and heterogeneous materials. The first application in the heterogeneous case was to organic molecules bound to a solid support, as in solid phase synthesis [50]. The spin rate requirements for such samples are less stringent than for solids, as the strong dipole–dipole interactions are already partly averaged by local motions. Further, attention is often focused on hetero-nuclei. For these, the dipolar interaction with protons is removed by rf decoupling and the chemical shift anisotropy (CSA) is the most important remaining interaction. Slow rotation and CSA combine to produce intense spinning side-bands which again move away from the centre band as the angular speed increases. Rates of a few kHz have proved sufficient for this purpose.

Just as organs, tissues, and membranes can be studied by HR-MAS, so can living cells [51–54], specially by its heteronuclear variants. A review has already been published [55]. Related techniques (so-called "slow" magic angle spinning, the PASS and PHORMAT experiments) allow much lower rotation speeds (1–40 Hz) and will be discussed in the next subsection. 1D experiments usually incorporate a Carr–Purcell–Meiboom–Gill spin–echo sequence to discriminate against broad signals. It is important that the pulses be synchronized with the rotor rotation in order to eliminate artefacts. Most known 2D/3D sequences can be adapted to the HR-MAS experiment. Furthermore, a pulsed magnetic field gradient may be applied along the rotation axis, allowing for shortened phase cycles. Li et al. [56] provide an exhaustive study of the effects of experimental parameters.

2.2.3. Magic angle turning

Most of the reported applications of HR-MAS mention that cells, even when spun at 4 kHz, remain, for the most part, viable. There are, however, reports to the contrary [52,57]. A further concern is the spinning of pathogenic bacteria in rotors that are liable to shatter. There seems to be no accepted safe protocol for the HR-MAS investigation of pathogens. There are thus strong incentives to develop methods that would allow much lower spinning rates. These methods exist; as the subject has recently been exhaustively and authoritatively reviewed [58], we will limit ourselves to a few mainly historical remarks. Magic angle turning (MAT) methods, as they are sometimes called, stem from a long series of developments: the TOSS and PASS sequences of Dixon [59], the magic angle hopping method of Bax and co-workers [60], the continuous turning sequence of Gan [61] extensively refined in the group of Grant [62,63], and the work of Antzutkin et al. [64]. Two experiments, PASS and PHORMAT, have been applied by Wind and co-workers in particular to living cells. Both are 2D experiments. PASS (spin rate ≈40 Hz) yields an ordinary spectrum in the t_2 dimension and a stack of spinning side-bands in the t_1 direction, while PHORMAT (spin rate ≈2 Hz) yields the usual (isotropic) spectrum along f_1 and the chemical shift anisotropy along f_2. Wind and Hu [58] report an investigation of *Shewanella oneidensis* in a dense suspension or attached to quartz beads; metabolic activity could be followed under good conditions.

2.2.4. Microprobe

Sensitivity considerations are just as important in solid-state as in liquid-state NMR, and several of the same recipes have been applied. A recent development is the work of Sakelleriou and co-workers [65] who have designed a probe in which the rf coil is wound on and around a capillary tube containing the sample, thus achieving a nearly unity filling factor. This assembly is then centred inside a commercial rotor. The coil is inductively coupled, without material contact, to the rest of the circuit. The resulting sample volume is about 25 nL.

2.2.5. Frequency and intensity calibration

Calibration of solid-state spectra is difficult. The samples are often heterogeneous mixtures of liquids and solids. There is no generally accepted inert and miscible reference compound. Chemical shift referencing is usually achieved with an external reference, a capillary mounted coaxially in the rotor. Here, magic angle spinning has an advantage over the usual liquid-state external referencing: the bulk susceptibility correction comprises a $3\cos^2\theta - 1$ orientation factor which vanishes under MAS conditions. There only remain solvent shifts which can be easily accounted for, as shown in a careful study by Morcombe and Zilm [66].

Intensity calibration raises even more questions. For absolute calibration, an external reference is again used, which will be spatially separated from the sample and will therefore experience different rf fields. In contrast to solution work, where concentration ratios are sought, relative peak areas may not reflect relative material amounts. For this reason, solid-state specialists refer to "spin counting" (beware that the same phrase is also used in a different meaning, namely the determination of spin or atom connectivities using multiple quantum coherences). The ERETIC method, in which an externally generated rf signal provides the intensity reference, affords a promising alternative [67]. Further difficulties are associated with differences in spin dynamics between reference and the various sample constituents. In order to obtain valid quantitative data from a ^{13}C CP-MAS spectrum, one should, in principle, determine T_1^C, the carbon spin–lattice relaxation time, $T_{1\rho}^H$, the proton rotating frame relaxation time, and T^{CH}, the inverse of the cross polarisation rate constant. Simple single pulse experiments (the so-called direct polarisation method) are often closer to being quantitative. These issues are discussed in detail by Maciel and co-workers [68,69].

We end this section by mentioning a very practical issue, the filling of rotors. Hanoulle et al. [70] have described a useful protocol to fill a rotor with a cell paste, which is summarized in Fig. 3.

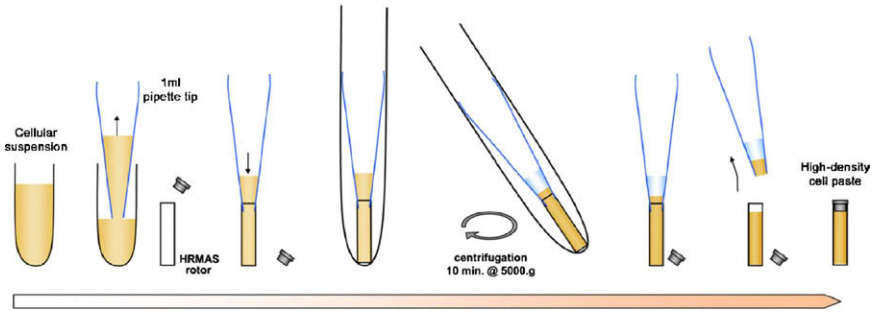

Fig. 3. A schematic of a convenient procedure to fill HR-MAS rotors. Centrifugation is used to pack cells in the rotor. Reproduced from [70].

2.3. In vivo systems

Because NMR spectroscopy is a non-invasive technique, it is possible to perform *in vivo* experiments on living microorganisms in the magnet. This approach presents several advantages: (i) One can monitor the experiment in real time so that decisions can be made to modify or improve the protocol. (ii) When a kinetic run is recorded, all spectra are collected on the same sample. In addition, this procedure is much cheaper when labelled compounds are used. (iii) Some species or parameters can be only monitored under *in vivo* conditions and are lost when working on extracts, namely macromolecules such as glycogen, polyphosphates and other intracellular polymers, and transmembrane gradients (Na^+ and H^+). Of course some drawbacks are also incurred with *in vivo* NMR: (i) Signals are quite broad (30–50 Hz) because of the heterogeneity of the sample. Consequently some information is lost due to the lack of resolution, for instance sugar–phosphate signals usually overlap. (ii) The intracellular metabolite concentration is the limiting factor that determines the sensitivity of the experiments because it cannot be increased by freeze drying or purification procedures. (iii) The presence of intracellular paramagnetic ions carried by oxidative enzymes (Mn^{2+} and Fe^{2+}) can modify T_2 values in such a way that no spectra can be recorded with intact cells. (iv) Specific set-ups must be devised to maintain cells alive.

The NMR nuclei most frequently studied are ^{31}P and ^{13}C, but some papers describe *in vivo* NMR with ^{23}Na, ^{1}H, ^{19}F, ^{2}H, and ^{15}N. Classical high resolution NMR spectrometers with vertical magnets can be used except that NMR tubes are usually wider (10–20 mm diameter tubes) to allow a larger volume of cells to be studied thus increasing the sensitivity. Some probes are dual $^{13}C/^{31}P$ probes that can switch from one nucleus to the other. The question of reference for chemical shift or quantification is usually solved by the introduction of a capillary, centred in the sample tube, containing a suitable compound (for instance benzene for ^{13}C, NaCl for ^{23}Na, dimethylphosphonate for ^{31}P, etc.). The cell density used is quite high (10^9–10^{11} cells/mL) in order to monitor kinetics within rather short times (from a few minutes to a few hours). Decoupling sequences must be chosen to avoid heating of the biological material (MLEV, WALTZ, etc.).

Two main types of devices can be used to perform *in vivo* NMR and maintain cells under physiological conditions: they can be just supplied with gas (O_2 or CO_2 for aerobic or anaerobic conditions), or they can be perfused with a liquid medium. A few examples of such devices are presented below.

2.3.1. Non-perfused systems

2.3.1.1. Systems for anaerobic cells. Although the handling of anaerobic cells, especially strictly anaerobic cells, is difficult outside the magnet, it becomes an advantage for NMR studies. Indeed, because cells are rather densely packed in the tube they already are under strong anaerobiosis; furthermore, it is only necessary to work under CO_2 and close very tightly the tube in order to maintain anaerobiosis. Fig. 4 presents a system used for ^{13}C NMR studies of the strictly anaerobic rumen bacterium, *Fibrobacter succinogenes*.

2.3.1.2. Systems for aerobic cells. Working with aerobic cells is more difficult because an airlift system is needed to inject O_2 continuously. In that case the presence of paramagnetic O_2 and also of bubbles may induce a loss of homogeneity of the field. The airlift system originally described by Santos and Turner [71] was used in many

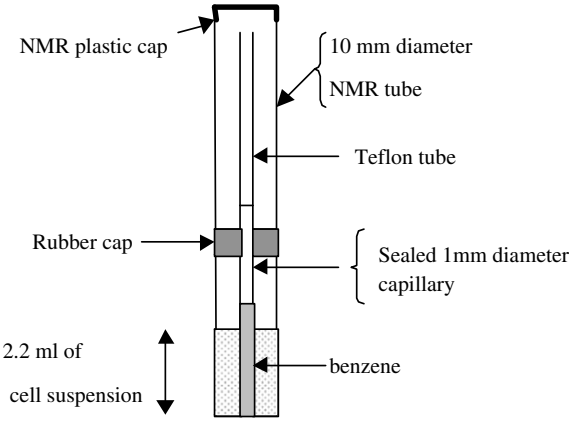

Fig. 4. ^{13}C *in vivo* NMR experiments: 10 mm diameter NMR tube containing a 1 mm diameter sealed capillary filled with pure benzene. Bacterial cells are under a CO_2 atmosphere to ensure strict anaerobic conditions.

studies by this group [72–74]. A more recent system is presented in Fig. 5 [75]. A special device was developed to study mycelium/pellet forming organisms such as *Aspergillus terreus* by Lyngstad and Grasdalen [76].

2.3.1.3. Bioreactors. Because of the low sensitivity of NMR, *in vivo* systems require high cell densities to avoid long measurement times. However, generally cells are harvested to be concentrated and sample preparation can disturb cell physiology. To overcome this problem, the group of de Graaf [77,78] designed a membrane cyclone reactor suitable for *in vivo* NMR measurements to enable growth of microorganisms within the magnet at high microbial cell densities. A detailed scheme of this reactor and its insertion in the NMR spectrometer are presented in Fig. 6(a) and (b), respectively. This system was successfully applied to ^{31}P and ^{13}C NMR studies of *Zymomonas mobilis*, *Corynebacterium glutamicum*, and *Saccharomyces cerevisiae*. Although this system is extremely interesting, it is not commercially available and no other laboratory has used it. Other types of bioreactors are those of Noguchi [79] in which a cell suspension circulates to and from the sample tube and of Rijhwani [80] and Gmati [81] where the culture is entirely contained in a mini reactor inside the spectrometer. A pH sensitive electrode may be inserted in the large sample tube.

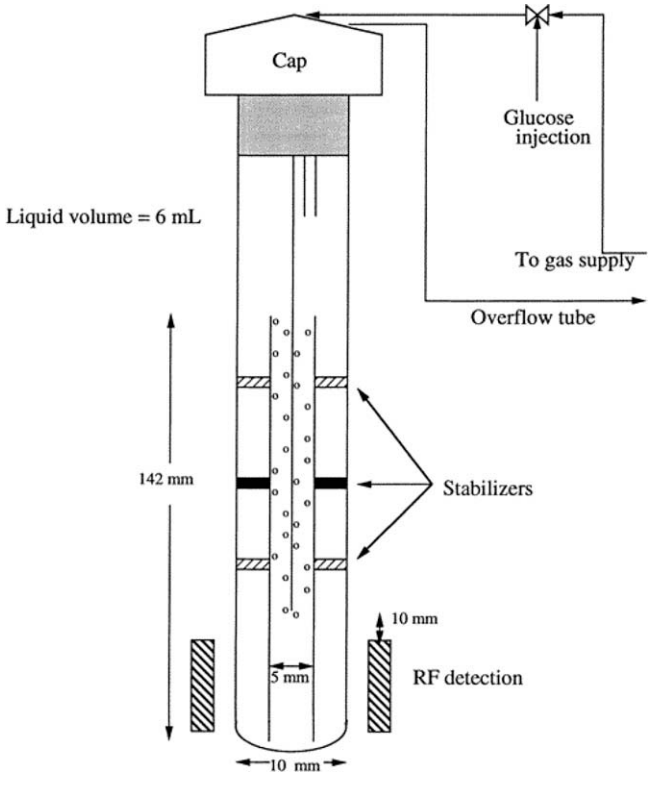

Fig. 5. Schematic of an internal loop airlift reactor for NMR spectroscopy experiments. Gas enters through the capillary tubing creating circulation of the solution up the draft tube and back down outside the draft tube. The draft tube is held in place by plastic stabilizers. The cap is fitted with an overflow tube as a safety precaution. The injection port for glucose is at a connection point in the tubing leading to the gas supply (after Melvin and Shanks [75]).

2.3.2. Perfused systems

The circulation of a physiological medium with controlled pH and gas concentration can allow cells to survive for a long time (a few days) in the magnet. Metabolites do not accumulate in the medium in that case. In addition, various experiments can be made on the same sample just by changing the composition of the medium. However this type of device is trickier to operate because pumps and tubes are needed and can fail.

To avoid the simultaneous circulation of cells with the perfused medium, these may be immobilized in a solid matrix. Different systems of immobilization are used such as agarose gels [82–86], alginate beads [87] and silica gels [88]. Fig. 7(a) shows an example of a perfusion set-up used for studying *Aspergillus niger* [87]. With this system, cells embedded in alginate beads showed a much longer life than simply packed mycelium. Fig. 7(b) presents an alternative system used to study *E. coli* cells embedded in agarose beads [86].

3. In-cell NMR

The phrase "in-cell NMR" refers to the NMR study of macromolecules, protein structure, protein dynamics, and protein interactions inside a cell. Here, the microbe provides its cytosol or periplasm as a biologically relevant solvent/medium. Protein concentrations inside cells are usually too low to allow NMR observation. Early work in this area included the following. Observation of several protons of myoglobin or haemoglobin in red cells or organs is made possible by the very high concentration of these proteins in red cells and by the fact that their resonance frequencies are contact-shifted outside of the normal range (see [89] and references therein). In particular the binding of oxygen could be followed through the chemical shift of a histidine proton. Aromatic amino acids ring substituted by fluorine can be incorporated in proteins by the cell machinery. Such ^{19}F-labelled molecules revealed an important fact: the viscosity of the cytosol is only about twice that of water [90], raising the hope that high resolution studies would be possible given sufficient sensitivity.

Steady progress in NMR sensitivity and advances in molecular biology now allow the recording of multidimensional NMR spectra of macromolecules in cells. Gronenborn and Clore demonstrated that high quality HSQC spectra could be obtained from cell extracts [91]. Lippens and co-workers [92,93] implemented both solution and solid-state (HR-MAS) methods to record the spectrum of a cyclic glucan in the periplasmic space of *Ralstonia solanacearum* (a phytopathogenic bacterium). In this system, normal 1D proton spectra are irreproducible and generally poorly resolved. On the other hand, HR-MAS spectra are reproducible and much better resolved. They can be further