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EDITORIAL TO THE SPECIAL ISSUE

Amphipols: Where from? Where to?

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I am often asked how the idea of amphipols came about. Now, exactly 20 years later, is perhaps a good time to recount the story. My main field of expertise is membrane biochemistry. The French *Centre National de la Recherche Scientifique* (CNRS) was originally designed by its founders as a multidisciplinary research organization. Until its recent splitting into several institutes, it offered a particularly favorable environment for scientists of different backgrounds to develop original projects at the interface between their respective fields. In the fall of 1990, Paul Rigny, then director of its Chemistry Department, launched a series of meetings around the theme of "Organized molecular systems", among which membranes feature prominently. Three interdisciplinary round-tables took place that year (chemistry/physics, chemistry/biology, biology/physics), followed, in 1991, by a big meeting in Bordeaux. The meeting itself was very formal and, to me at least, rather boring, but the round-tables were an excellent opportunity to build bridges between communities. I took part in the latter two, and was so interested that I became involved in many kinds of activities at the interfaces between biology, chemistry and physics. I coorganized an international summer school in 1994 in Cargèse (Corsica), along with the physicist David Bensimon and the chemist Ludovic Jullien, and took part in or set up various interdisciplinary networks of French laboratories dealing with selforganizing systems, as well as a large CNRS interdisciplinary granting project. In the process, I was exposed to lots of information that have only tenuous connections to biology (including the marvelous properties of clays). Among those were some intriguing stories, such as the immiscibility of hydro- and fluorocarbons, or the fact that surfactant micelles can be used to crosslink hydrophilic polymers carrying sparse alkyl chains, with spectacular effects on their rheology. A few years and many shared beers later, some of these systems found their way into membrane biology. The fluoroalkane story developed into a long and friendly collaboration with the chemist Bernard Pucci, of the University of Avignon, with whom we designed and validated a series of fluorosurfactants for handling membrane proteins. The polymer story led to amphipols.

In 1994, I was approached by Roland Audebert, from the *Ecole Supérieure de Physique et de Chimie Industrielles* (ESPCI, Paris), a chemist active in the field of amphipathic polymers. Roland had heard me present talks about membrane proteins and their complicated and often contentious relationship with detergents. His idea was to use them as nodes to cross-link hydrophobically modified polymers. Roland knew that some membrane proteins undergo conformational changes upon binding a ligand or detecting light and, although I don't remember whether we discussed this point or not, I feel certain that he anticipated this could possibly lead to "intelligent" materials that would react to specific stimuli, a subject of great interest to him. I was not particularly thrilled by the idea of using my precious, hard-to-prepare membrane proteins to make gels, but I was keenly interested in exploring alternatives to detergents and their denaturing properties. I countered with the suggestion to make very small and flexible amphipathic polymers that could replace detergents at the surface of membrane proteins, thereby keeping them soluble and,

I would like to dedicate this special issue to the memory of *Annemarie Weber* (1923–2012), inspiring teacher and lifelong friend—J.-L. Popot.

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perhaps, stabilizing them. I distinctly remember telling Roland, towards the end of a three-way meeting with Christophe Tribet, a young chemist trained in the physical chemistry of poly(amphiphiles) who was about to embark on a post-doctoral stint with the two of us, that there was a 90 % chance that mixing membrane proteins with such polymers would yield "a gooey product of no use to anyone", but that I was willing to take on the risk. The ESPCI crew must have realized immediately that this was a win–win proposition for them: should my suggestion actually work out, the three of us might open up a whole new field; should it fail, the "gooey product" was exactly what Roland had been looking for originally. As for me, I was ready to give it a shot, even if I felt it to be a very long one.

In the following couple of years, Christophe joined my laboratory to familiarize himself with membrane biochemistry—he co-authored in 1997 a nice article on the (in)stability of the cytochrome $b_6 f$ complex in detergent solutions—went to Roland's lab to synthesize and purify the polymers we had settled for (much shorter and much more densely grafted with alkyl chains than had been studied before), and then back to the IBPC to test them on our pet membrane proteins of the time, bacteriorhodopsin and the $b_6 f$ complex. Much to our surprise, the approach worked immediately, in the sense that membrane proteins depleted of detergent in the presence of what we dubbed 'amphipols' remained soluble and reasonably monodisperse even when centrifuged in surfactant-free sucrose gradients. It was an exhilarating period, when every experiment we designed worked as well as we could hope for. In the following years, of course, as generally happens in science, fine-tuning the chemistry, understanding the solution properties and developing the applications of amphipols turned out to be a more complex, grinding, frustrating, and, to sum it up, classical research project than these first months had led us to expect—much to the despair of Yann Gohon, the first Ph.D. student to tackle amphipols. Yann nevertheless sailed out of these treacherous waters with flying colors. Roland, unfortunately, died prematurely, in 1997. Christophe kept up the good work—focusing more on amphipol/membrane interactions and excitable polymers—and we kept happily collaborating whenever a mutually interesting subject came up. On the chemistry front, we were soon joined by Bernard Pucci, who undertook to develop non-ionic amphipols, a protracted story that has only recently yielded really satisfying molecules, and later by Fabrice Giusti, originally from Bernard's laboratory, who integrated ours to create labeled, functionalized, or chemically different amphipols. Other types of amphipols have been developed in other laboratories as well.

Funding this project has not been a peaceful walk in a rose garden. Except for the CNRS, its technology transfer branch, and a handful of excellent laboratories with whom we set up fruitful collaborations, the support of the French scientific community was timid—to put it euphemistically. Some of it was actually closer to the kind of support the rope extends to the hanging man. As an anecdote—hopefully to be meditated by decision makers—our first application to the *Agence Nationale pour la Recherche* proposing to try to fold G protein-coupled receptors using amphipols, which was based on preliminary data with bacteriorhodopsin, was rejected on the ground that is was "neither new nor original", when no such attempt had ever been carried out before. The following year, the same application, now buttressed by highly encouraging data obtained on a leukotriene receptor, was criticized as being "unfeasible", and would have been turned down again, for exactly opposite reasons to those used the previous year, but for the courage of a single dissenting examiner. There was some consistency in this pattern, but not of the scientific type. As late as 2008, as two dozen papers had already been published and applications were flourishing, the CNRS official in charge of overseeing the organization of my succession at the head of our laboratory recommended to the director of the Life Sciences department that the whole project be abandoned, as it had, in his informed opinion, "come to a dead end". (My advice had not been sought.) It took what diplomats call "a frank exchange of views" one evening in the office of the director for this insightful suggestion to be dropped. In this difficult context, the funding initially obtained from the CNRS and the European Community was short-lived. The decisive impetus came neither from France nor from Europe, but from a daring grant awarded to us in 2000 by the *Human Frontier Science Project Organization*, which I will never thank enough for the trust they placed in us. Without them, the project would have died in its infancy from lack of support, and there is little doubt that, today, no membrane biologist would use amphipols. In the following decade, development was mainly supported, with ups and downs, against very strong winds, and at the cost of preposterous heaps of useless paperwork, by the European Community, until enough data had finally been accumulated to build confidence in the methodology and give it a solid foothold.

The first article describing amphipols was published in 1996. Over the 18 years that have elapsed since then, an enormous amount of work has been invested, in two scores of laboratories, to study and diversify them, to understand their behavior, and to develop their applications. This progress has been reviewed periodically and is the subject of an update in the present special issue of *J. Membr. Biol.* It is, of course, very difficult to offer an unbiased view of a field with which one is so closely associated. It is my impression, however, that the selection of reviews and research papers offered here confirms that amphipols have

joined nanodiscs, lipidic mesophases and a few other innovative approaches as one of the accepted novel tools that membrane biochemists and biophysicists can turn to when facing too many difficulties using detergents. The contributions gathered here range from hard physical chemistry to vaccinology, structural biology to proteomics, in vivo biodistribution studies to molecular dynamics, etc. Most studies of membrane proteins classically carried out in detergent solutions can in fact be advantageously performed after trapping them with amphipols, and new applications emerge every year, the development of many of them helped by the multifarious resources of polymer chemistry. The long shot we risked back in 1994 has matured into a widening family of useful tools. Having reached retirement age, I am now turning to other pursuits, but I hope to still be around ten or twenty years from now to discover, with great curiosity, what the field will have morphed into by then. In the meanwhile, I dearly hope that the interdisciplinary spirit that sparked this project will be revived at the CNRS and, there and elsewhere, will spur other unconventional endeavors.

Paris, August 11, 2014.