

death. Finally, nerve biopsies of the patient, who suffered severe axonal loss, showed that surviving Schwann cells are morphologically (TEM) and functionally aberrant (electrophysiology). This prompted the physicians to provide the patient with high doses of *N*-acetylcysteine and this new treatment is associated with a clear improvement in motility.

A second project was driven by a forward mutagenesis screen in *Drosophila* in which we previously identified variants in the fly *Ankle2* gene. We discovered that loss-of-function mutations of the human ortholog of *ANKLE2* cause microcephaly in a child [3]. We then discovered, in collaboration with Nevan Krogan and Priya Shah, that a Zika virus protein, NS4A, interacts with and inhibits *Ankle2* [4,5]. Our studies in *Drosophila* combined with human genetic studies allowed us to uncover a pathway that implicates several genes associated with primary microcephaly in human.

We identified five additional microcephaly patients with variants in *ANKLE2* and showed that these variants act as loss-of-function alleles in flies. Our data show that *Ankle2* is an ER-localized protein essential for proper ER and nuclear envelope structure. Mutations in *Ankle2* affect cell division, spindle alignment, and localization of asymmetric determinants including the proteins of the Par complex. *Ankle2* strongly interacts with the nuclear kinase Ballchen, the homolog of human VRK1, an established microcephaly locus. *Ankle2* mutants fail to maintain Ballchen/VRK1 in the nucleus, and we propose that this leads to a “gain-of-function” phenotype where Ballchen/VRK1 can ectopically interact with targets that it normally does not interact with. This results in severely reduced aPKC phosphorylation, which has previously been shown to have reduced kinase activity. The *Ankle2* pathway also physically and genetically interacts with L(2)gl, an inhibitor of aPKC activity, which is consistent with the observed aPKC defects noted in *Ankle2* mutants. Finally, we show that expression of NS4A, which binds to and inhibits the function of *Ankle2*, phenocopies *Ankle2* mutant defects in neuroblasts, and these defects can be rescued by modulation of the *Ankle2* pathway [3] (Link et al., 2019).

Our work highlights an important pathway required for proper human brain development: *ANKLE2* and VRK1 are both associated with microcephaly; a member of the Par complex has been linked to brain defects in mice; and LLGL1, the homolog of L(2)gl, maps to the critical region of Smith Magenis Syndrome, a disease associated with microcephaly. Furthermore, we identified many novel variants associated with microcephaly in many of the human homologues of the fly genes described in this study.

Disclosure of interest The author declares that he has no competing interest.

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Drosophila research: From the genome to the proteome

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Starting from 1900, *Drosophila melanogaster* has been studied in the laboratory by scientists interested in many different aspects of animal development, physiology, and evolution. Over much of this more than hundred year period, genetic analyses have been at the basis of most studies, also those who led to the Nobel prizes attributed to scientists working with *Drosophila*. More recently, fluorescent proteins and optogenetic tools have been added to the ever-expanding genetic toolbox allowing for a better understanding of basic cellular processes underlying complex developmental processes.

Even more recently, a novel approach is being added to the toolbox. Small protein binders can be used to directly target and manipulate proteins in their native environment, in cells of the living organism. The development of numerous antibody- and non-antibody-based scaffolds of protein binders (Fig. 1) has allowed the rapid identification of such small binding domain, recognizing virtually any target protein of interest. Such binding molecules can then be functionalized in many different ways, allowing for acute and direct protein manipulation

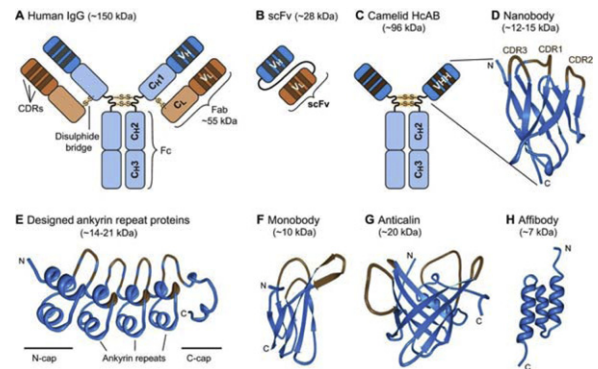


Fig. 1 A–H: Different protein binder scaffolds that can be used in developmental biology studies (see [1]).

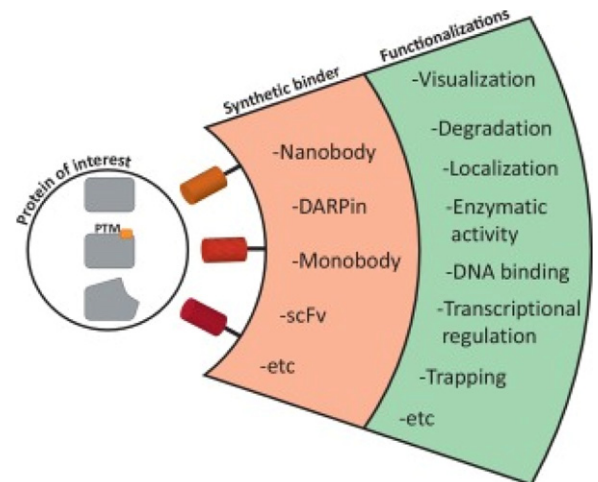


Fig. 2 Protein binders can be expressed as functionalized proteins in vivo (see [2]).

in vivo (degradation, trapping, relocalization, etc.; Fig. 2). Several cases in which such small, functionalized protein binders have been used in *Drosophila* will be discussed, and a look into the promising future of research with *Drosophila* will end the presentation.

Disclosure of interest The author declares that he has no competing interest.

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Insects as interactants in artists' minds: Symbols and anti-symbols

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Insects, as small animals of frequent and easy access to humans, at least under their non-flying larval stages, have always been of interest to our species. The interest has probably been nutritional, but also naturalistic and symbolic. Many aspects of insects' biology are of high symbolic potential, among which biodiversity, metamorphosis or flight. These three creative attributes of the insect orders, over the mammalian order at least—the *self* or reference class of human life—, probably induced early interests in human societies. This interest is indicated by folk classification of insects in some indigene cultures of northern America or central Africa [1]. Representation of insects in paleolithic art is scarce but existing; one of the earliest attested records could be that of “the buprestid” of Arcy-sur-Cure, revised as a ground beetle–carabid–(Magdalenian III, ≈ 15000 years BP), or that of “the grasshopper” of Enlène [2]. The first is sculpted in lignite and could have served as a shamanic representation, while the latter is more obviously naturalistic in nature, and carved on bone. The more recently discovered Chauvet cave might contain insect representations (a “butterfly” or a many-legged animal), but no attested insects yet. Similarly, no insect mention come yet from Sulawesi caves (late Mousterian, < 30,000 years BP).

Neolithic representations are much more numerous, as exemplified by bee hunting and bee rearing in Iberic or Egyptian parietal and monumental arts. This trend culminated in Egyptian jewellery and its quasi-industrial representations of scarab or dung beetles. Noteworthy, most prehistoric representation of insects lie on coleoptera and Hymenoptera, and associated with symbolic features linked to colour, strength (beetles), metamorphosis (all) or social and utility behaviours (bees, ants). Aristotle himself did not clearly recognize aphids as a

group, as the ancient Greek word ψυλλίδα did not differentiate aphids and psyllids. Many mentions however, on the reproduction of such insects, for example, show that he was talking of aphids.

In line with these historical roots, insects have been associated with far eastern or Christian society arts [3]— in contrast, Islamic and Jewish imaging or alimentary taboos having banned them from visible representations in middle eastern societies. A previous review on the subject gives an excellent outline of our matter, restricted to European painting [4]. I will here sketch a variant perspective, trying to uncover a hitherto hidden monument of cultural entomology, with the case study of the representation of aphids (Hemiptera: Aphididae) in various arts and all periods of history. Our purpose is to draw a reflexive perspective on what such invisible insects, through their invisibility, tell us of their representation in artists' minds, and more generally in any human mind (being he a reader, a writer, a watcher, an actor, or in any position of the artistic universe).

The first and archetypal position to be quoted in my survey of invisible insects is the *detail aphid*. This position of a subject, the detail, have been analysed thoroughly in the history of arts [5]. It reflects both scientific and artistic mastery and precision (it is painted because *it exists*), and a position of power of the painter who is freely choosing low signification or even forbidden or non-codified subjects due to the act of freedom of the artist (and art factories, often specialized in such very specific matters); I paint it because *I want/know* it. This is characteristic of the Flemish and Dutch renaissance, as visible in the *Bouquet of flowers in a vase* by Jan velvet Brueghel the Elder (Fig. 1). In contrast to many of its fellow insects, cricket, coleoptera, syrphid, the aphid is extremely difficult to localize (Fig. 1A), and needs an intensive focus on its lily host-plant to be seen (Fig. 1B). However, in spite of its minute size, it is precisely depicted, and the *crescent-marked lily aphid* was recognized by expert aphidologists (Colin Favret, *pers. comm.*). A non-expert comparison between object and subject (Fig. 1B,C,D), be it living (1C) or mounted (1D) is edifying and informs us on the naturalistic and observational abilities of the painter(s).

As one may imagine, such identification of aphids in European classical painting is extremely difficult and has been only found on three occasions and with the help of the G-art Gigapixel project, apart from Fig. 1: in another still life by velvet Brueghel, *Flowers in a Vase* displaying at the Antwerp museum of fine arts, and in a third *Chat renversant un vase de fleurs* by Abraham Mignon, Museum of Fine Arts, Lyon.



Fig. 1 *Bouquet of flowers in a vase*, by Jan Brueghel the Elder (1608, oil on copper 65 × 45 cm); original (A) in the Pinacoteca Ambrosiana (Milan, Italy), © with permission. Detail with an aphid on a lily leaf (B) situated at the bottom-left corner of the bouquet. Inferred species, with the help of Dr C. Favret: *Neomyzus circumflexus*, quoted from Encyclop'aphid (C) with its typical darkish crescent on the back (D).