Where is the field of α-helix mimetics going?

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The formation of stable or dynamic protein complexes is pivotal in virtually all biological processes. As a consequence, the loss or misregulation of essential protein-protein interactions (PPI), as well as aberrant protein self-assembly, underlies many human diseases. Although PPI systems were initially considered high-risk targets due to relatively large and flat interaction surfaces, the fact that small segments of the interface (hot-spots) might contribute to high-affinity binding opened the opportunity of PPI modulation with secondary structure mimetics and small-molecules [1].

α–Helices are the most common secondary structure elements within proteins, and almost 60% of PPI sites involve an α–helix within hot-spots [2]. Therefore, it is no surprise that considerable efforts have been devoted in recent years to develop molecules able to compete with α–helices in therapeutically relevant PPI. Different ways to fix or to mimic α–helix conformations for targeting PPI have been described, including cyclic and stapled peptides, β-peptides, peptoids and the most recent non-peptide α–helix mimetics.

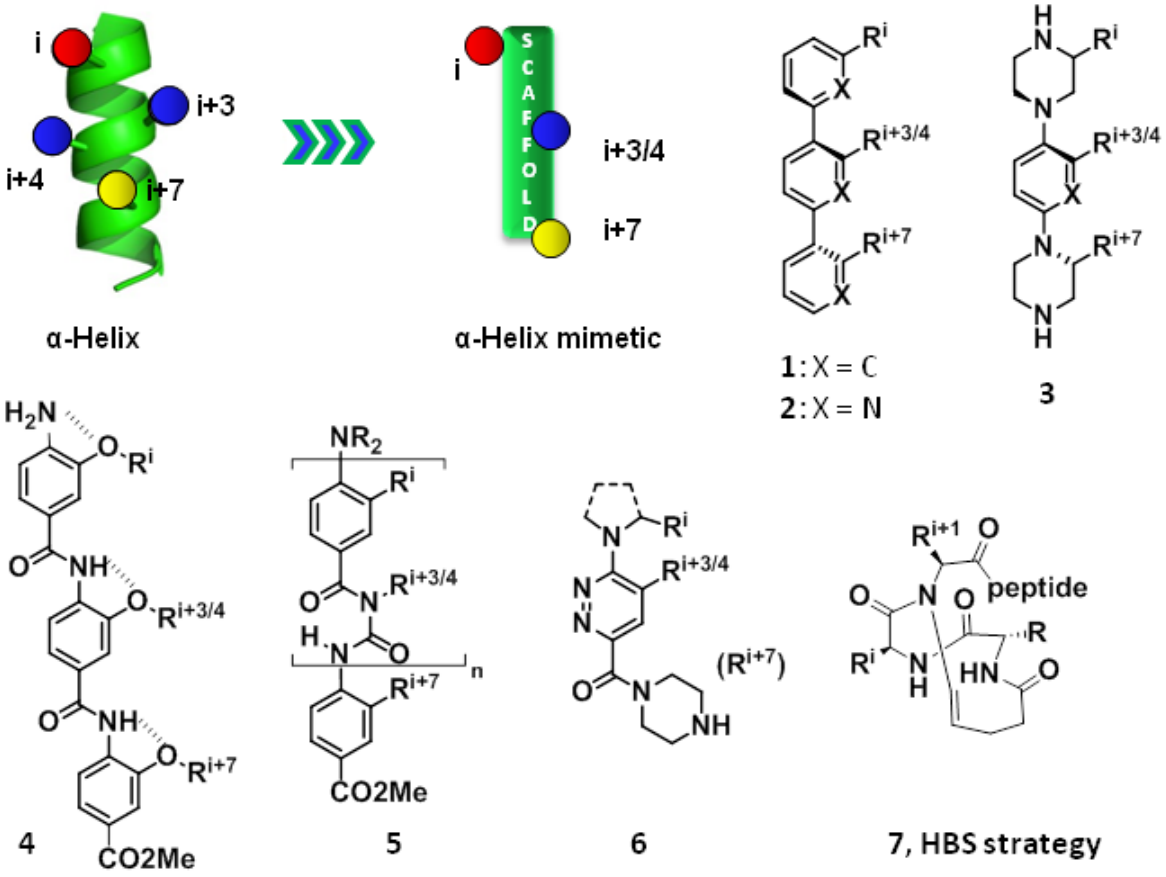

Figure 1. *Translation of α–helix peptides into α–helix mimetics and representative examples*

The seminal work by Hamilton's group about the terphenyl scaffold (**1**), able to correctly situate i, i+3 (i+4) and i+7 side-chains of the α–helix, has been followed by other related central frameworks with improved solubility, such as oligobenzamides and ureas, and different heterocycle-containing analogues (**2-5**) [3,4]. The rationale for the design of these non-peptide α–helix mimetics and some representative examples are depicted in Figure 1. The synthesis of these mimetics usually follows tedious, linear strategies, but some modular synthetic routes, which could facilitate the future preparation of libraries, are appearing [5,6]. As an example, researchers at Pfizer have described the preparation of pyridazine-based α–helix mimetics (**6**) able to bear a variety of amino acid side-chains, through C-C, C-N and C-O bond forming reactions and starting for accessible synthetic intermediates [6]. The application of this methodology to therapeutically relevant targets has not been published yet.

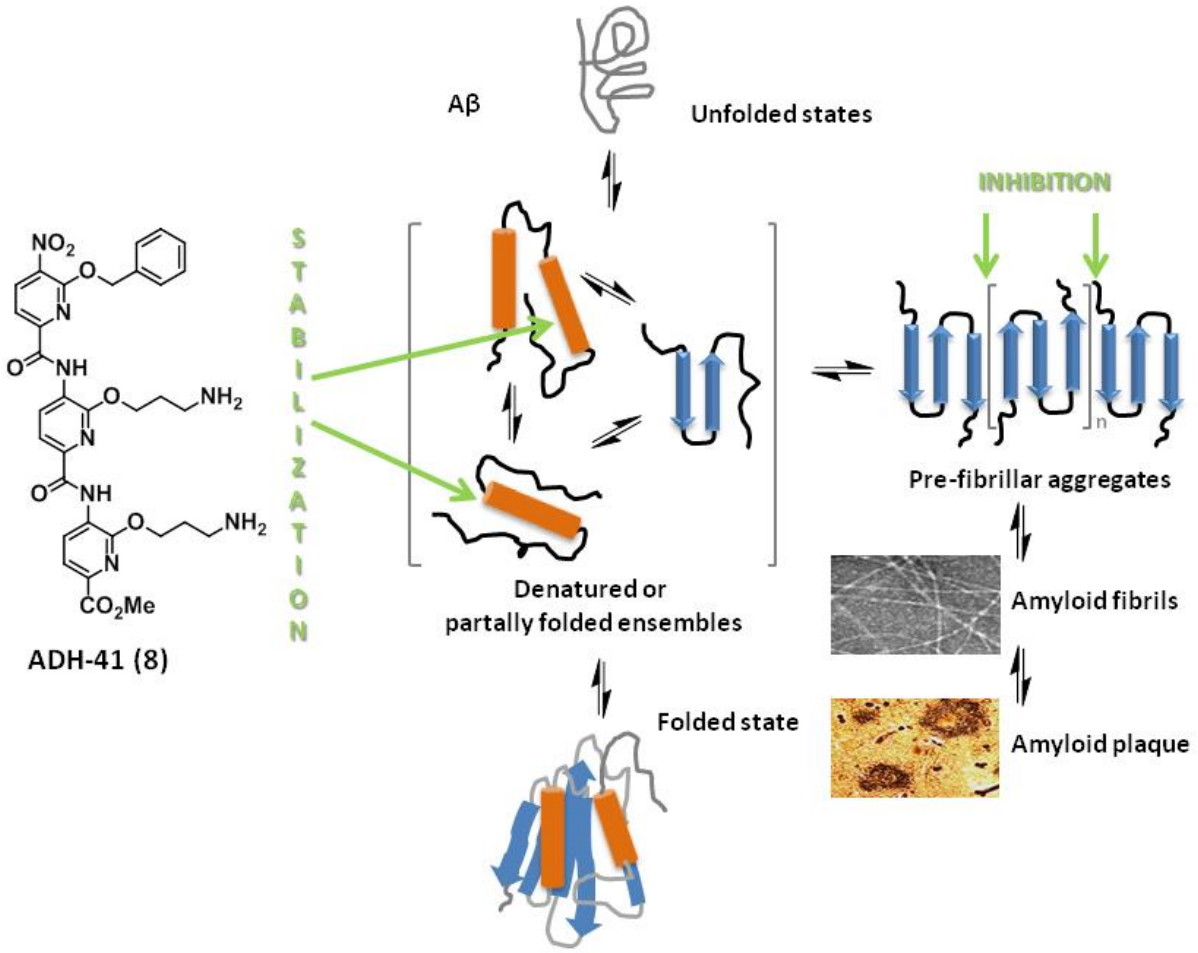

Figure 2. *Alteration of prefibrillar Aβ formation with a designed non-peptide α-helix mimetic*

A recent successful example of a totally non-peptide α–helix mimetic for the modulation of amyloid β peptide (Aβ) self-assembly is illustrated in Figure 2 [7]. The oligomerization of Aβ, first as β–sheet fibers and then into amyloid plaques, is a central feature of Alzheimer's disease (AD) progression. Recent experimental works established that pre-fibrillar soluble oligomers are the most neurotoxic species, while accumulation of plaques led to presynaptic loss and neuronal death. Within this system two main strategies for therapeutic intervention can be distinguished: a) destabilization of prefibrillar formation, and b) stabilization of specific Aβ conformations to alter oligomerization to toxic species. Aβ peptides mainly adopt random-coil conformations in aqueous solution, but can promote also specific 3D arrangements under different conditions, including α–helices with K16, V18, E22 and D23 residues exposed to solvent. Based on this hypothesis, the group of Hamilton synthesized and evaluated a series of oligopyridylamide α–helix mimetics in an attempt to change the Aβ solution behavior [7]. One of their compounds, ADH-41 (**8**, Figure 2), binds to Aβ with low micromolar affinity, induces α–helical conformation of the amyloid peptide, as deduced by CD and NMR experiments, and more importantly is a potent antagonist of Aβ fibrillation, being considered a lead compound toward new agents for AD treatment.

Most terphenyl-like mimetics display the functional groups corresponding to a single –helical face, but about 40% of the high affinity PPI helices interact with its partner through two of three faces. The strategy in these cases is to work with stabilized peptides, either through side-chain to side-chain cyclic peptides, including the so called stapled peptides, or using the Arora's hydrogen bond surrogate approach (HBS, **7**, Figure 1) [2], all of them allowing the synthesis on solid-phase.

As an alternative approach to mimic the α–helix secondary structure, the groups of Wilson and Aitken developed α/β/γ-foldamers capable of adopting 12,13-helix conformations (Figure 3). Using the 2-aminocyclobutanecarboxylic acid as a fundamental scaffold, they prepared a series of metabolically stable foldamers able to inhibit the p53/hDM2 interaction, a PPI critical for stress-induced cell cycle arrest and apoptosis [8]. Under hypoxia or DNA damage, the p53 protein induces the transcription of genes involved in cell-cycle control, and apoptosis. In the absence of stress, HDM2 (human double minute 2) interacts with p53down-regulating its activity. Tumor cells often overexpress HDM2, leading to a loss of cells primary response to stress, and resulting in unchecked cell growth. Therefore compound **9**, as well as other inhibitors of the p53/hDM2 interaction derived from the p53 helix on the hDM2 binding domain, could be important for oncogenic treatment after appropriate optimization.

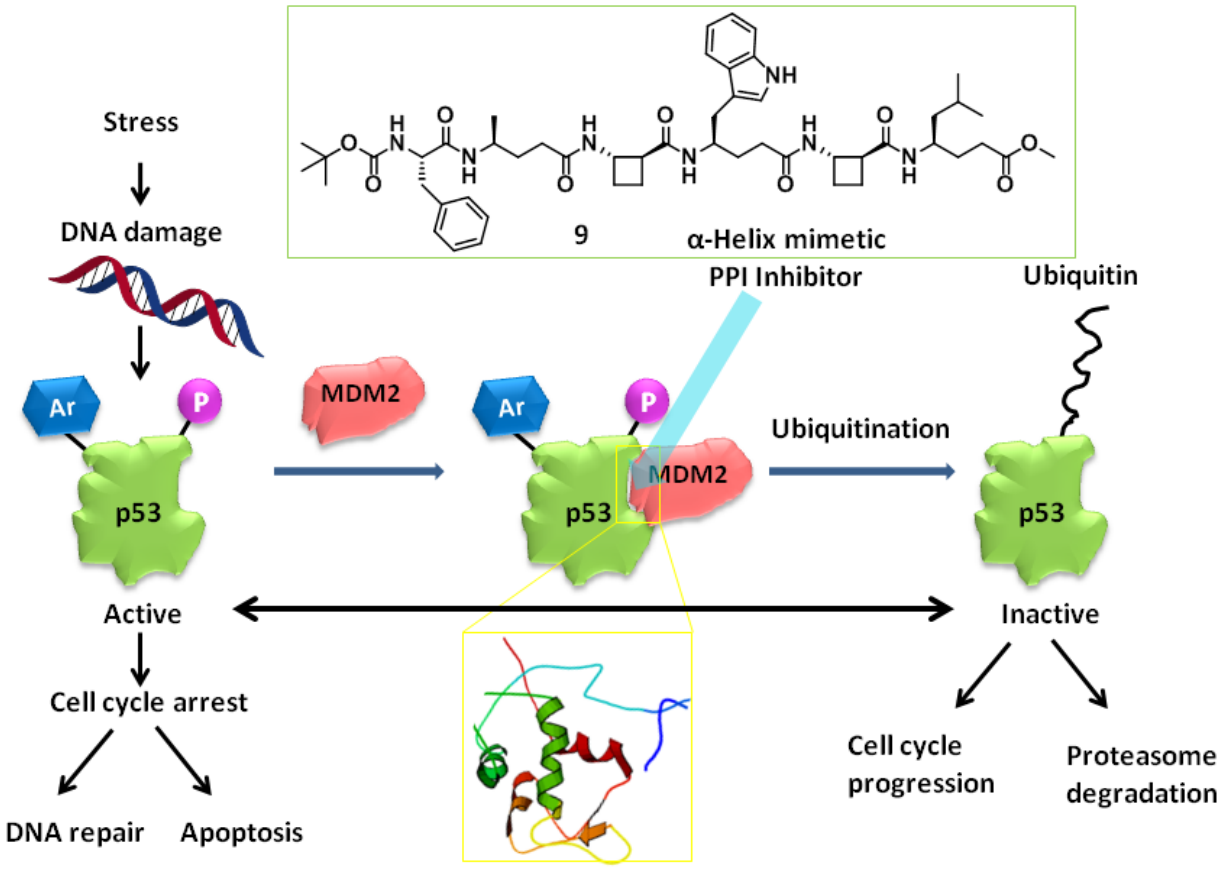

***Figure 3**. Inhibition of p53/hDM2 interaction through helical foldamers*

The growing interest in the development of α–helix mimetics is demonstrated not only by the continuous publications in scientific journals, but essentially by the appearance in the last five years of a number of patents that claim their potential therapeutic uses [9]. Despite the achievements so far by academic and industrial teams, new efforts are needed in the field, both to advance in the design of innovative, non-peptide compounds capable of mimicking more than one helix faces, and in the development of straightforward, easy synthetic procedures that could facilitate the generation of α–helix mimetic libraries for HTS screening. Considering that there are more than 30.000 PPIs of therapeutic interest and the high incidence of α–helices within their contact interfaces, there is still considerable potential for new developments. Medicinal-chemists and solid-phase synthetic experts have still much to contribute in this area.

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[9] For representative recent patents in the field, see: **2012**: WO2012021144 , WO2012097133; **2013**: WO2013123511; **2014**: US20140051706 , US20140256817,WO20140 61824 ; **2015**: US20150011728 , WO2015127342 , WO2015179547; **2016**: US9255086; WO2018008436, WO2016010210.