Towards "bionic" proteins: replacement of continuous sequences from HIF-1α with proteomimetics to create functional p300 binding HIF-1α mimics†

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Using the HIF-1α transcription factor as a model, this manuscript illustrates how an extended sequence of α-amino acids in a polypeptide can be replaced with a non-natural topographical mimic of an α-helix comprised from an aromatic oligoamide. The resultant hybrid is capable of reproducing the molecular recognition profile of the p300 binding sequence of HIF-1α from which it is derived.

Nature uses a stunning array of architectures to carry out complex tasks including catalysis and cell signalling. Biopolymers are complex to prepare these feats because they self-organise and present functional motifs (e.g. an active site or binding surface) through precise 3-D orientation of primary structure. A long term goal in chemical and synthetic biology, therefore, is to expand on this diversity and, through the incorporation of non-natural functionality, elaborate bio-macromolecules with enhanced or orthogonal functionality and/or properties.1 Foldamers are oligomers that adopt well-defined conformations and either replicate structural and functional features of natural biomacromolecules or access, using non-natural building blocks, novel folds and functions not found in nature. An alternative approach to this bottom-up strategy, termed “protein-prosthesis”, lies at the cross-roads with strategies to chemically2–4 or genetically4–6 introduce non-natural function into proteins. In protein prosthesis,9,7 backbone engineering8 allows individual residues or sequences within proteins to be replaced with non-natural residues.9–13 Notable examples include the incorporation of β-amino acid residues in the B1 domain of Streptococal protein G (GB1)14 and Betabellin-14,15 the re-engineering of a heterodimeric chorismate mutase enzyme16 using sequence based design, the replacement of loop regions in GB1 using PEG17 and the incorporation of an entire β-amino acid topological mimic of an α-helix into ILR.18 α-Helix mimetics19–23 employ a suitably functionalised generic scaffold to reproduce the spatial projection and composition of key side chains found at a helical interface between two proteins. Such α-helix mimetics have been shown by us24,25 and others26–28 to act as potent inhibitors of protein–protein interactions, but there are limited studies on their incorporation into higher-order structures.29 In this manuscript we illustrate the first steps towards this goal by replacement of an entire segment of the HIF-1α transactivation domain with an aromatic oligoamide (Fig. 1). In doing so, we provide the first example of a replacement of an extended peptide sequence with a topographical mimic.

Our design focused upon a minimal region of the HIF-1α C-terminal transactivation domain (C-TAD) which interacts with the CREB-binding protein (CBP)/p300 co-activator to promote transcription (Fig. 1a).30,31 Previously our group illustrated that two helical domains (HIF-1α794–826, i.e. helix 2/3) within the 42 residue C-TAD peptide (HIF-1α794–826) were necessary for measurable binding to p300 (Fig. 1c).32 We had also identified a helix mimetic 1 based on our 3-O-alkylated aromatic oligoamide scaffold which was designed to mimic helix 3 of the HIF-1α C-TAD (HIF-1α7816–826) and shown to act as a low μM (IC50 = 9.2 ± 0.9 μM) inhibitor of the HIF-1α/p300 interaction (Fig. 1b).33 In our design (hybrid 2), the helix 3 region (HIF-1α7816–826) of HIF-1α (HIF-1α7816–826) was replaced with the previously identified helix mimetic33 and the remainder of the sequence preserved (Scheme 1a). In parallel, we also designed a series of helix 2/3 conjugates 3a–c linked by PEG spacers (Scheme 1a); the purpose in designing these compounds was to ascertain the extent to which the p300 binding potency might be maintained when the linker region between key helical regions (HIF-1α794–808 and HIF-1α7816–826) was replaced.

Previously developed solid-phase synthesis methods for the preparation of oligobenzamidates facilitate rapid preparation of helix mimetic trimers34 but we found the method unsuitable for preparation of the target peptide-helix mimetic conjugate 2.

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To address the poor nucleophilicity of trimeric oligoamides with a terminal aniline, Fmoc-glycine was coupled to an isobutyl monomer 7 using dichlorotriphenylphosphorane (Scheme 1b). Noteworthy in this sequence was the use of an allyl ester, which facilitated ester cleavage in the presence of the base-labile Fmoc protecting group. Fischer esterification of nitro acid 6 using dichlorotriphenylphosphorane (Scheme 1b).

One explanation that might account for this observation is that there may be a non-specific component to PPI inhibition as supported in previous studies.38 This indicates chelate co-operativity for 3a–c here and validates the approach.

At first the lower inhibitory potency of both HIF-1α/p300 and hybrid 2 in comparison to helix mimetic 1 (9.2(±0.9) μM) which we determined previously39 might seem counterintuitive. One explanation that might account for this observation is that as the length of the PEG linker in 3a–c increases, so does binding affinity. In spite of the weaker binding affinities observed for 3a–c, the weak binding for HIF-1α/p300 (helix 2) and HIF-1α/p300 (helix 3) in isolation and lack of allostERIC co-operativity between the two observed previously32 indicates chelate co-operativity for 3a–c here and validates the approach.

The hybrids 2 and 3a–c were then tested in a previously described fluorescence anisotropy p300/HIF-1α competition and p300 binding assays respectively (Fig. 2a).33 Hybrid 2 was shown to inhibit the interaction with comparable affinity to the HIF-1α/p300 peptide from which it was derived (IC50 values hybrid 2 = 83 ± 1.8 μM; HIF-1α/p300 = 89 ± 2.8 μM).32 Crucially, to avoid disulfide formation, the helix mimetic peptide 2 conjugate bears a cysteine to serine modification (underlined in Scheme 1), whereas the peptide does not. The mutation of this cysteine residue to a serine has been shown to reduce affinity by approximately 10-fold for the native sequence,36 which should be considered when comparing the affinities. It should also be noted that this affinity does not derive solely from the peptidic region, as a peptide comprising the same residues (HIF-1α/p300) shows no inhibitory activity (IC50 > 500 μM).32

The PEG linked hybrids 3a–c exhibited lower affinity for p300 (Kd = 144(±13) μM, 3a/b > 200 μM (Fig. 2c) – than FITC-HIF-1α/p300 (Kd = 6.74 ± 0.54 μM).32 This suggests either that the linker is not sufficiently long or that the side chains from the linker between helices 2 and 3 make productive non-covalent contacts with p300. A requirement for optimal linker length is supported by the fact that as the length of the PEG linker in 3a–c increases, so does binding affinity. In spite of the weaker binding affinities observed for 3a–c, the weak binding for HIF-1α/p300 (helix 2) and HIF-1α/p300 (helix 3) in isolation and lack of allostERIC co-operativity between the two observed previously32 indicates chelate co-operativity for 3a–c here and validates the approach.

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acid residues from the HIF-1α sequence to the core helix mimetic 1 in hybrid 2, the specificity and selectivity of binding towards target interactions was improved.

In conclusion, we have described the first example of a peptide-helix mimetic hybrid and in doing so conceptually illustrated that extended sequences from proteins can be replaced with molecules that topographically mimic such sequences. Our immediate efforts will focus on structural and biophysical studies on these hybrid mimetics with a view to optimizing the binding properties. Future efforts will focus on incorporating such secondary structure mimetics into longer protein sequences and exploring this replacement strategy for a broader array of protein functions. Application of this generic approach for preparation of functional peptide-helix mimetic hybrids, could allow assembly of protein-like objects with enhanced properties e.g. proteolytic and thermal stability, married with
superior recognition properties (e.g. selectivity) when compared with simpler proteomimetics.

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