VIP Synthetic Transcription Activator

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A Cell-Permeable Synthetic Transcription Factor Mimic**

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There is great interest in the development of methods with which to control the expression of specific genes in living cells and animals. Over the past several years, the development of RNA-interference (RNAi) technology has provided a relatively general way to knock down the expression of any gene.[1] However, there exists no corresponding method by which to upregulate gene expression specifically, although this would also be of value as a tool for biological research.[2–4] Furthermore, reagents capable of specific activation of gene expression could potentially be interesting as a new class of therapeutic compounds. Although engineered proteins have shown promise in this application,[5,6] there are many advantages to the development of cell-permeable synthetic transcription factor mimics (STFMs). Most strategies towards this end have focused on linking a sequence-specific DNA-binding molecule to a moiety capable of binding to a transcription coactivator or some other transcription protein.[7] This type of chimera should be able to mediate the recruitment of the transcriptional machinery to the promoter to which it is bound, thus mimicking one of the fundamental properties of the DNA-binding and activation domains (AD) of native transactivator proteins.[8]

Herein we report a cell-permeable STFM **1** (Scheme 1) that activates gene expression in living cells. This chimera contains a DNA-binding hairpin polyamide[9,10] linked to a derivative of a coactivator-binding peptoid (oligo-*N*-substituted glycines[11]) isolated from a combinatorial library. We demonstrate that this molecule is capable of activating the expression of a reporter gene with several polyamide binding sites in the promoter by fivefold over the basal level in mammalian cells without the use of transfection agents of any kind. We further demonstrate that this compound activates the expression of a number of endogenous genes with multiple polyamide binding sites in their promoter region. This work demonstrates the feasibility of achieving a general strategy for cellular gene activation with synthetic molecules.

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As will be described in detail elsewhere (manuscript in preparation), a combinatorial library of approximately 50000 hexameric peptoids was screened for ligands against the core KIX (KID-interacting; KID = kinase-inducible domain) domain (residues 586–672) of murine CREB binding protein (CBP; CREB = cAMP response element binding protein),[12] a mammalian transcriptional coactivator. Six "hits" were isolated and several derivatives of these peptoids were analyzed for their KIX affinity and cell permeability. It was found that the carboxyfluoresceinated peptoid **3** (Scheme 1), a truncated derivative of one of the hits, binds a glutathione *S*-transferase (GST)–KIX fusion protein with a K_d value of 11.6 μM as determined by fluorescence polarization (Figure 1). A similar titration experiment with GST showed no evidence of binding (data not shown), indicating that the peptoid binds to the KIX domain. As a control, the scrambled carboxyfluoresceinated peptoid **5** does not bind GST–KIX (data not shown).

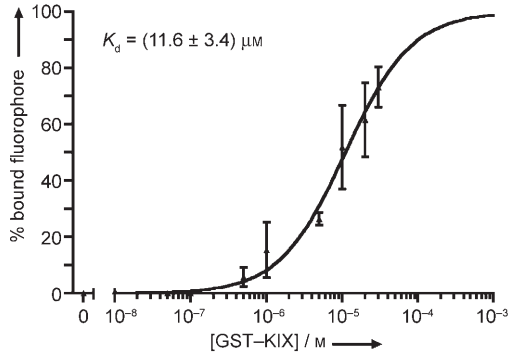
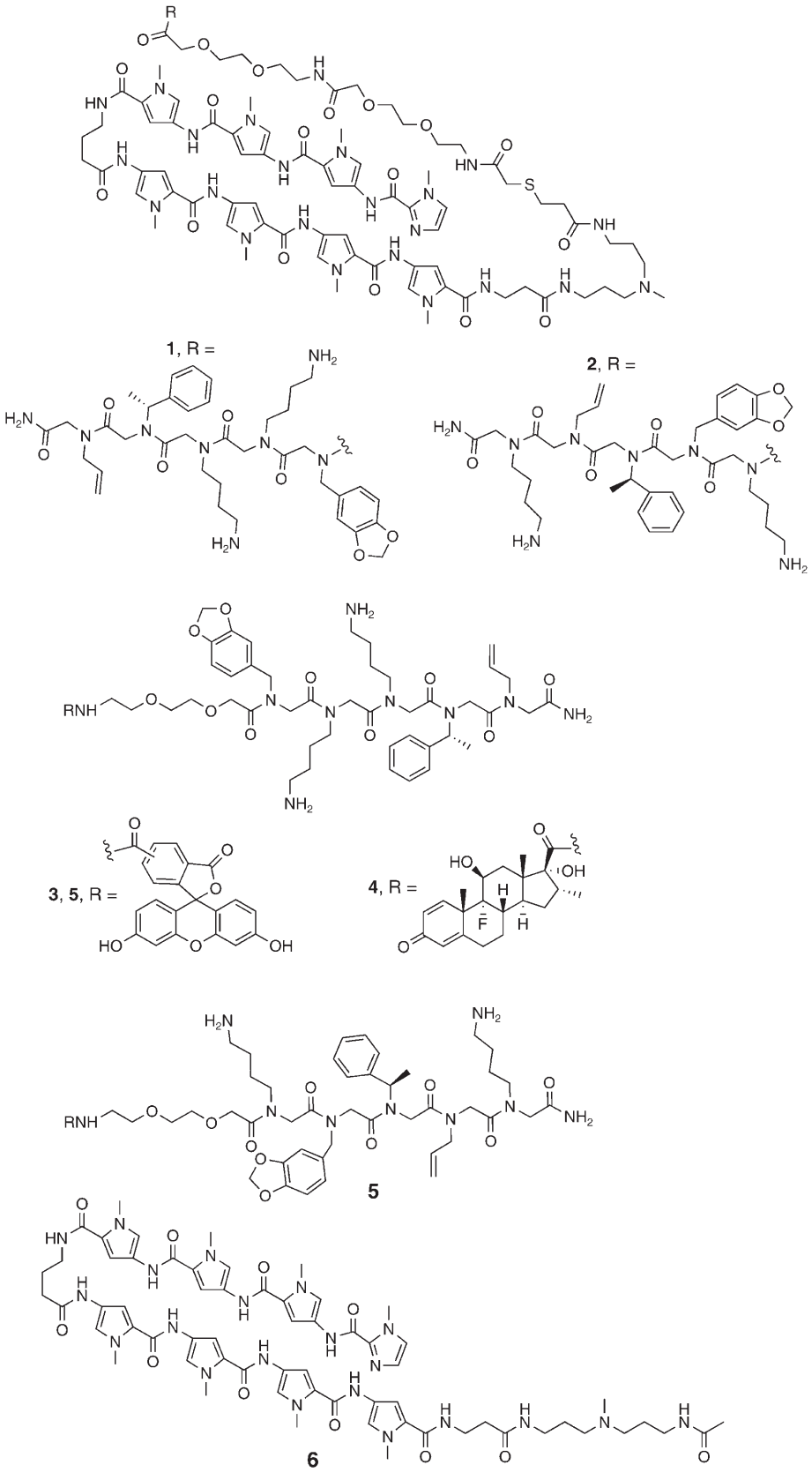


Figure 1. Binding of carboxyfluoresceinated peptoid **3** to GST–KIX as monitored by the increase of fluorescence polarization upon the addition of protein.

The peptoid was then conjugated through a flexible linker to the DNA-binding hairpin polyamide ImPy7,[9] which is designed to bind the sequence 5′-WGWWWW-3′ (W = A or T[13]), to give compound **1** (Scheme 1). To ensure that the conjugate can still bind to DNA as well as the KIX domain, a biotinylated DNA template with six ImPy7 binding sites (whose sequence is from the plasmid pGL3-6 × HPB; HPB = hairpin polyamide binding site, see below) was immobilized on a streptavidin agarose resin after incubation with various compounds and GST–KIX (Figure 2). In the presence of compound **1**, GST–KIX was recruited to the DNA template, indicating that both DNA–ImPy7 and peptoid–KIX binding interactions are retained. On the other hand, no GST–KIX was detectable in the presence of control compound **2** (a conjugate similar to **1** but with a scrambled peptoid sequence), **4**, or **6** (see Scheme 1 for structures).



Scheme 1. Structures of the compounds used in this study (see the Supporting Information for their synthesis).

After it was established that compound **1** can bind both DNA and KIX in vitro, we investigated whether it can activate gene transcription in living cells. Therefore, compound **1** was added to HeLa cells that had previously been transiently transfected with a plasmid-borne luciferase reporter gene containing six ImPy7 binding sites in its promoter (pGL3-6×HPB, see Figure S1 in the Supporting Information for sequence information).[14] A dose-dependent increase in luciferase expression was observed (Figure 3), with the maximum effect being a fivefold increase in luciferase expression at an extracellular polyamide–peptoid concentration of 3.0 μM. No activation was seen in various control experiments with compounds lacking the DNA-binding ImPy7 (compound **4**) or the peptoid AD surrogate (compounds **2** and **6**). Furthermore, incubation of chimera **1** with cells transfected with reporter plasmids that contained no ImPy7 binding site (pGL3-E1b) or two ImPy7 binding sites (pGL3–2×HPB)[14] in the promoter region also failed to show gene activation (Figure 3). These data demonstrate that activation of the reporter gene requires both the presence of multiple copies of the peptoid AD surrogate and its delivery to the promoter of the reporter gene through sequence-specific ImPy7–DNA interactions.

The data in Figure 3 demonstrate that reporter gene activation is possible with this compound, but the ultimate goal in this field is the activation of endogenous chromosomal genes. To determine if the hairpin polyamide–peptoid conjugate displayed activity on any endogenous genes, a genome-wide transcription profiling experiment was carried out. HeLa cells lacking the reporter plasmid were treated with either compound **1** (3.0 μM) or the polyamide without the peptoid AD mimic (compound **6**, 3.0 μM, see Scheme 1) for 40 h. Total messenger RNA was isolated from the treated cells and the

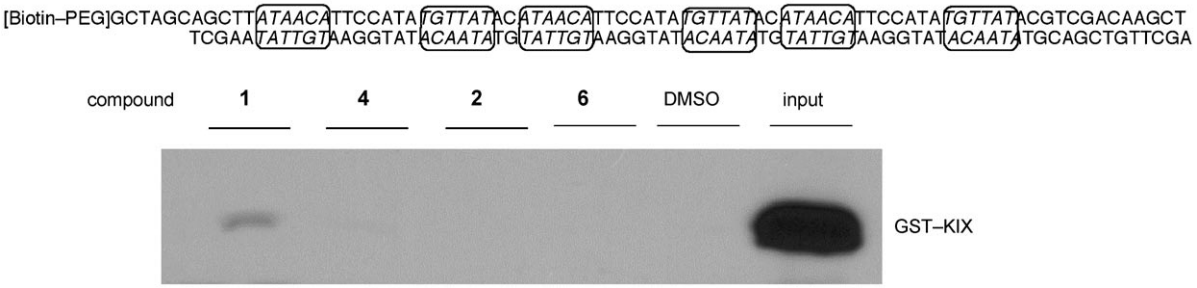


Figure 2. Compound **1** recruits GST–KIX to a DNA fragment containing six ImPy7 binding sites (boxed). The biotinylated DNA template was incubated with GST–KIX in the presence of **1**, **2**, **4**, **6**, or just the vehicle alone (dimethyl sulfoxide (DMSO)). Streptavidin agarose resin was then added and the bound protein was analyzed by Western blot with anti-GST antibody.

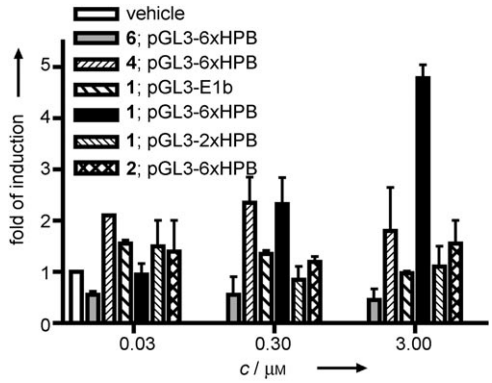


Figure 3. Transcriptional activity of STFM **1** in HeLa cells. The reporter plasmids pGL3-6×HPB, pGL3-2×HPB, and pGL3-E1b have six, two, and zero ImPy7 binding sites upstream of the luciferase gene, respectively. Compound **4** was prepared to study its cell permeability,[15] but is used herein as a control. The fold of induction represents the ratio of the inducible luciferase activity normalized to the constitutively expressed *Renilla* luciferase activity.

levels of approximately 46000 transcripts were compared to identify genes that were upregulated at least threefold in the cells treated with **1** relative to those treated with **6**.[16–18] This analysis identified 45 genes (see Table S1 in the Supporting Information). To determine if this activation likely reflected the binding of multiple polyamide–peptoid molecules to the promoters of these genes, these regions (defined here as from 1–1000 base pairs from the 5′ terminus of the transcriptional start site) were searched for sequences fitting the polyamide target site (5′-WGWWWW-3′). All these genes contain multiple ImPy7 binding sites (5′-WGWWWW-3′) in their promoter regions (see Table S1 in the Supporting Information). This is consistent with the idea that the STFM **1** is capable of activating endogenous genes by binding to promoters with multiple target sites. We also recognize that there exist genes that were not activated more than threefold by this compound, but contain several potential polyamide binding sites in their promoters, possibly because of the presence of repressive chromatin structure on these genes. Clearly, understanding the many complexities of the binding of polyamides to endogenous promoters in living cells will constitute an important challenge as this field moves forward.

The results presented herein represent the first example of a cell-permeable synthetic transcription factor mimic[19] that activates gene expression in living cells. We stress that this result was achieved without artificially facilitating entry of the compound into the cells by transfection or in any other fashion. Although another polyamide–synthetic AD surrogate has been reported[7] with activity in vitro, this molecule did not activate gene expression in living cells, apparently owing to insufficient permeability. A comparison of the levels of reporter gene activation achieved by using constructs containing zero, two, or six polyamide binding sites revealed that several copies of the STFM **1** must bind to the target promoter to achieve significant activity (Figure 3). This is also consistent with the results of the genome-wide transcription profiling experiment (see Table S1 in the Supporting Information).

Along with previous studies from our laboratory,[20,21] these data validate peptoids as promising AD surrogates for the construction of STFMs. In this earlier work, we identified a peptoid that binds a different region of CBP[21] and demonstrated that it has AD-like activity in cells although its activity in the context of an STFM has yet to be determined. Indeed, we are in the process of investigating whether this molecule and the peptoid reported herein could activate transcription in a synergistic fashion if delivered to the same promoter by attached polyamides. As peptoid libraries appear to be a rich source of ligands for many different proteins,[22] it may also be possible to isolate peptoid ligands for other types of transcription cofactors, such as chromatin remodeling complexes, again with an aim to achieve greater potency through synergistic effects. These efforts are underway and will be reported in due course.

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