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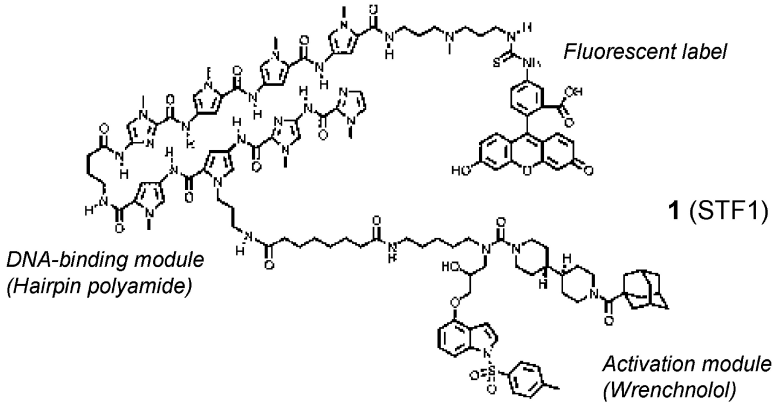

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Small Molecule Transcription Factor Mimic

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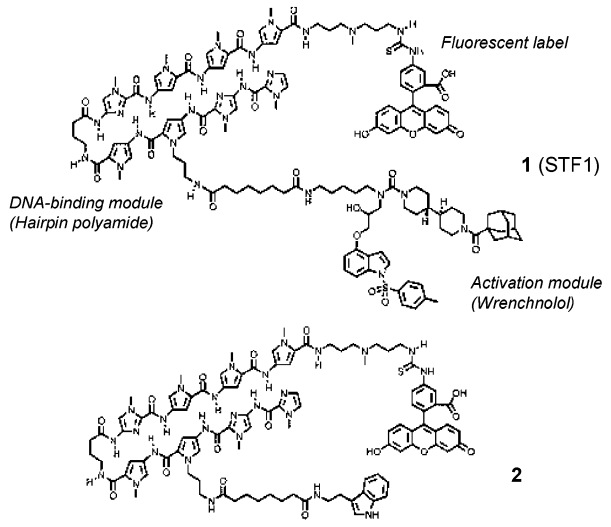
Regulation of gene expression by transcription factors touches many aspects of eukaryotic biology, and its systematic, external control by organic molecules represents a challenge in chemistry.[1,2] A number of attempts have been made to mimic transcription factors by synthetic molecules,[3−9] including those utilizing DNA-binding hairpin polyamide attached to peptide activation domains.[3,4] These studies indicate that it is possible to reproduce the gene-activation function of transcription factors by synthetic molecules. However, the presence of peptide regions in these molecules may limit their future applications. Here we report the design of a nonpeptidic small molecule that mimics a transcription factor. Naturally occurring transcription factors usually have two separable domains for activating selective genes: one for binding to specific promoters and the other for activating transcription through protein−protein interactions.[10,11] Our designed molecule **1** (STF1: *s*ynthetic *t*ranscription *f*actor 1) comprises two functional domains (Scheme 1): a hairpin polyamide molecule that binds specifically to 5′-TGACCAT sites in DNA[12] and a wrench-shaped synthetic compound (wrenchnolol) that binds to the Sur-2 protein,[13,14] a subunit of human mediator complex that links transcription activators to RNA polymerase II in human cells.[15,16]

Scheme 1



STF1 was synthesized by amide couplings of the hairpin polyamide, wrenchnolol, and a polyethylene linker. An internal pyrrole residue was chosen as an attachment site in the hairpin polyamide because previous studies had suggested that an extension at this position provides effective projection of an activation module.[4,5] The ability of STF1 to activate transcription was evaluated in vitro by using a reporter construct in which transcription of the reporter gene is controlled by six repeats of the hairpin-polyamide-binding sites (Figure 1). STF1 activated transcription of the reporter gene in a dose-dependent manner (lanes 2−5), whereas the control molecule **2** lacking the wrenchnolol moiety had no detectable activity (lane 6). Addition of excess amounts of a dominant negative Sur-2 protein fragment (lane 7) or immunodepletion of endogenous Sur-2 protein from nuclear extracts (lane 8) rendered the activity of STF1 undetectable, consistent with our previous observation that wrencholol binds selectively to Sur-2 protein. A reporter construct with point mutations in the hairpin-polyamide-binding sites was unresponsive to STF1 (lanes 9−11), suggesting a high degree of selectivity of STF1.

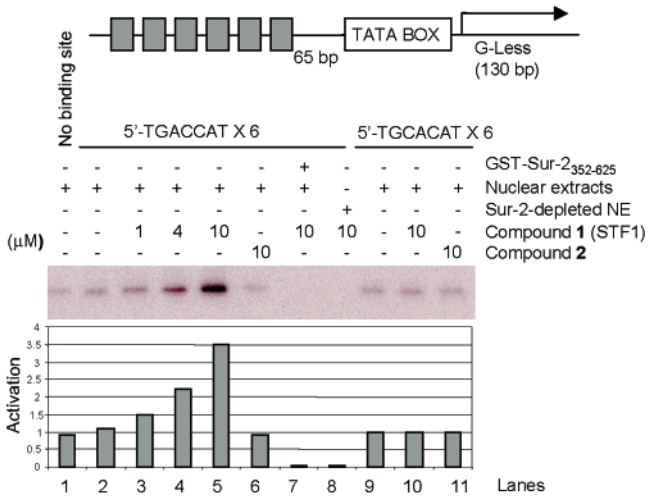


Figure 1. STF1 (**1**) activates transcription of a reporter gene in vitro. STF1 (0, 1, 4, or 10 μM) was incubated with HeLa cell nuclear extracts and a reporter DNA construct in which a G-less reporter gene is controlled by six repeats of the hairpin-polyamide-biding site (5′-TGACCAT) or those of its mutant (5′-TGCACAT). The 130-base mRNA of the reporter gene was detected by ^{32}P autoradiography and quantified by a phosphoimager. Addition of dominant negative Sur-2 protein fragment (GST-Sur-2$_{352-625}$) or immunodepletion of Sur-2 protein from nuclear extracts abolished the gene activation. It is evident that the reporter gene with mutated polyamide-binding sites is completely unresponsive to STF1 (**1**). Preparation of GST-Sur-2$_{352-625}$ and immunodepleted nuclear extracts is described in Supporting Information. A schematic diagram of the design of the reporter gene constructs is shown in the top. A gray square indicates a DNA sequence of the hairpin-polyamide-binding site.

To verify the biochemical mechanism of the STF1-mediated gene activation, the promoter region of the reporter construct was labeled with a biotin molecule and bound to an avidin−agarose resin after incubation with nuclear extracts (Figure 2). Western blot analysis of the bound proteins showed that no detectable levels of Sur-2 or RNA polymerase II were recruited to the promoter in the absence

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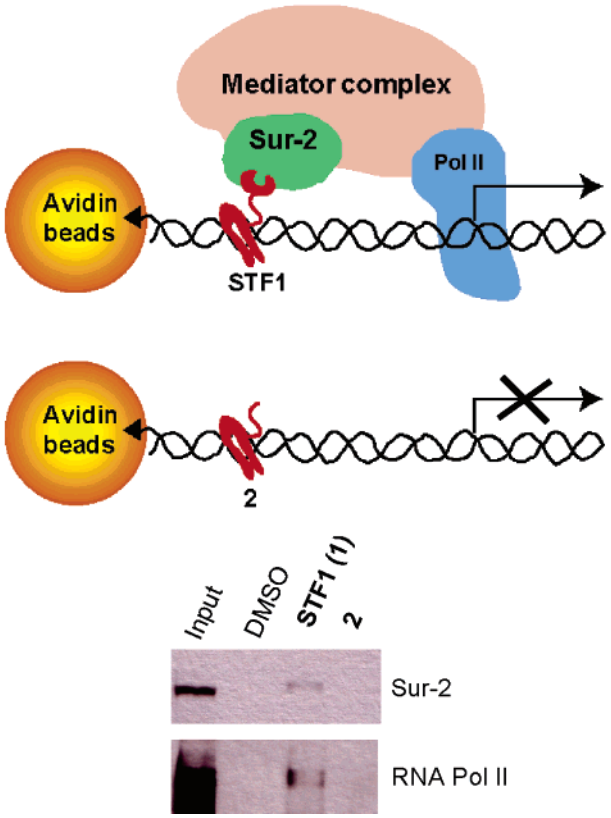

Figure 2. STF1 recruits Sur-2 and RNA polymerase II to the promoter. The promoter region of the reporter DNA construct was labeled by a biotin molecule and incubated with HeLa cell nuclear extracts in the presence of **1**, **2**, or solvent (DMSO) alone. The biotinylated DNA was recovered by using Neutravidin agarose, and the bound proteins were analyzed by Western blots with antibodies against Sur-2 and RNA polymerase II.

of STF1 or in the presence of the control molecule **2**. In the presence of STF1, in contrast, Sur-2 protein and RNA polymerase II were recruited to the promoter. These results support our notion that STF1 stimulates transcription by recruiting human mediator complex to the promoter through simultaneous contacts with Sur-2 and DNA.

Our results indicate that it is possible to generate a transcription factor out of nonpeptidic components.[17] The fluorescein moiety of STF1 permitted evaluation of its cell permeability.[12] Unfortunately, STF1 had limited cell permeability, even though the hairpin-polyamide−FITC conjugate and the wrenchnolol molecule are cell permeable as separate compounds.[1,12,14,18] By decreasing the size of the molecule and optimizing its physical properties, it may be possible to increase its cell permeability for biological studies.

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Supporting Information Available: Detailed experimental procedures. This material is available free of charge via the Internet at http://pub.acs.org.

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