Gene Expression Modulation Systems (GEMS): Optimized CRISPR-Based Epigenome Editing Platform for Versatile Epigenome Modulation

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Abstract

Epigenome editing holds immense potential for programmably modulating gene expression, enabling therapeutic applications. However there remain significant barriers to realizing this potential including in delivering bulky and highly active epigenome editing platforms *in vivo*. In this study, we present the development of a highly optimized CRISPR-based epigenome editing platform termed Gene Expression Modulation systems (GEMs).

Through extensive engineering efforts, we have made notable improvements in Cas proteins, guide RNA scaffolds, and added the discovery of novel compact modulator proteins. Our platform integrates these advancements to achieve precise and efficient control over gene expression. Firstly, we have systematically identified and characterized thousands of compact modulators capable of transient and persistent gene regulation.

These compact modulator proteins serve as effective tools for manipulating gene expression, allowing fine-tuning of specific target genes in a programmable manner. Additionally, we have engineered a CRISPR-Cas system to enhance the functionality of the ribonucleoprotein (RNP) complex for more efficient epigenetic editing. Through these modifications, we have achieved a more compact RNP complex that exhibits improved editing activity, thus enhancing the precision and efficacy of our epigenome editing system.

Overall, our GEMs provide a versatile and powerful tool for a broad range of epigenome modulation and gene therapy applications. The optimized CRISPR-based platform, combined with the extensive repertoire of compact modulator proteins, enables precise control over gene expression, opening new avenues for therapeutic interventions and potential cures for genetic disorders *in vivo*.

GEMS

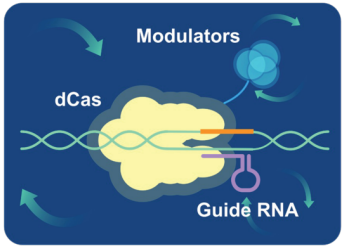


Figure 1. Gene Expression Modulation System (GEMS).

GEMS leverages the programmability of CRISPR systems and combines it with the power of epigenetics to precisely tune target gene expression. At the heart of the system is a suite of nuclease-dead Cas proteins (dCas) between 400 and ~500 amino acids in length (represented by the yellow blob in the schematic). At Epicrispr Biotechnologies we have devoted much effort to the discovery and characterization of compact proteins which we call Modulators. When fused to our dCas proteins, these Modulators are able to activate or suppress targeted genes in either a transient or durable fashion as desired. Modulators can be combined to derive novel activities as well as to produce synergistic target gene regulation. Lastly guide RNAs have been engineered to be both compact, and to enhance GEMS activities when complexed with the dCas-Modulator fusion.

As a consequence of the compactness and activity of each component of the GEMS platform, GEMS can be delivered to target organs *in vivo* for therapeutic applications.

Compact Cas Proteins

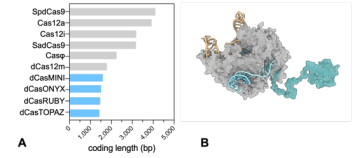


Figure 2. (A) Size comparison of Epicrispr Biotechnogies' Cas proteins to benchmark Cas molecules. CasONYX, CasRUBY, and CasTOPAZ all fall under 1500 nt in length (less than 500 amino acids). Together with CasMINI, they constitute a suite of compact and active proteins suitable for *in vivo*g ene therapeautic applications. **(B) Rendering of a representative Epicrispr dCas (grey) fused to a Modulator (in blue).**

Compact Modulators

Single Modulators

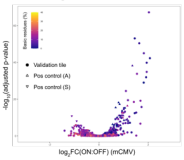


Figure 3. Single Modulators

At Epicrispr Biotechnologies we have conducted screens to successfully identify hundreds of Modulator proteins, capable of robust gene activation and suppression. These proteins, some of which out-perform benchmark activators (such as VP64 and VPR) and suppressors (such as Kox1 KRAB) are compact (less than or equal to 85 amino acids in length).

We have successfully identified biophysical features of these molecules that are key to their activity and exploit these features through semi-rational engineering approaches.

Combined Modulators

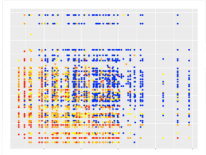


Figure 4. Combined Modulators

Rational combinations of transcriptional activation domains have long been used to demonstrate synergistic gene activation.

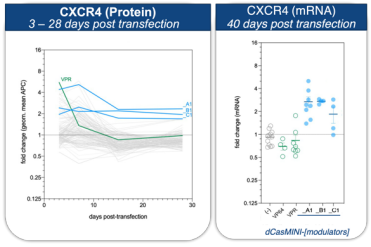
Here we have exploited this approach to combine hundreds of our Modulators in order to identify new activities, and identify synergistic combinations to potentiate target gene activation in human cells.

Machine-enabled Modulators

Figure 5. Machine-enabled Modulators

We have successfully developed a machine learning pipeline for the discovery of novel and active Modulator proteins. This has been accomplished by leveraging the data generated from our Modulator screens. By this approach both the scale of discovery and the rate of hit identification have been significantly enhanced (from 0.5% to 20%).

Epigenetic Modulators



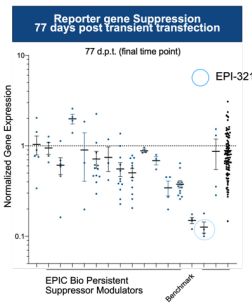


Figure 6. (A). Plot of CXCR4 protein expression in HEK 293 T cells transiently transfected with the indicated constructs. While benchmark activators like VPR (green line) show the anticipated initially robust activation of CXCR4, this activity decays over the course of 8-10 days post-transfection. This is anticipated as the initiating activity, VPR, is lost from the cells with subsequent rounds of mitotic cell division. By contrast, 3 Epicrispr Bio Modulators (A1, B1 and C1) in blue show sustained CXCR4 expression up to 30 days post transient delivery. **(B).** Messenger RNA from the same experiment was monitored at 40 days post-transfection. Corroborating the results from panel (A), transfection of Epicrispr Modulators resulted in gene activation 40 days post-transient transfection. **(C).** We have developed a compact and robust epigenetic silencing Modulator complex at Epicrispr Bio. In this assay, target reporter gene expression is significantly suppressed 77 days post-transient transfection. The Modulators showing the most robust activity (in blue circle) are those used in EPI-321 (described in Figure 8).

Epigenome editing *in vivo*

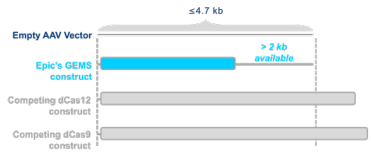


Figure 7 (A). Epicrispr Bio GEMS are ultracompact, mitigating AAV vector packaging constraints. All sequences required for the generation of GEMS *in vivo*, promoter sequences, linkers, dCas, modulator(s) and guide RNA are comfortably packaged within AAV vectors (represented by the blue rectangle in the schematic above), leaving space for additional therapeutic cargo.

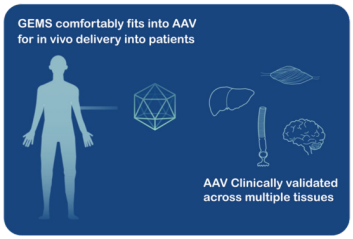


Figure 7 (B). Epicrispr Bio GEMS, packaged in AAV, can be delivered to multiple human tissues for *in vivo* therapeutics. GEMS exploits the most clinically de-risked platform for *in vivo* gene therapy to target multiple tissues in the human body. Epicrispr GEMS technology is poised to deliver first in class epigenome editing therapeutics in a clinical setting.

A potential cure for FSHD

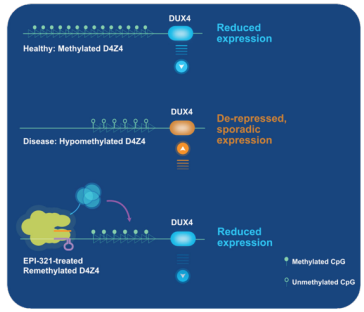


Figure 8. (A). A schematic representation of the epigentic state at the human D4Z4 locus on chromosome 4. The "healthy" locus is depicted showing methylated CpGs within the D4Z4 repeat region. Healthy individuals have >10 repeats. In diseased alleles there are typically fewer than 10 D4Z4 repeats and the region is typically hypomethylated at constituent CpGs. A documented consequence of this hypomethylation is the de-rpression of DUX4 gene expression. DUX4 is a developmental transcription factor whose aberrant expression in adult myocytes triggers a transcriptional cascade resulting in myocyte apoptosis. These molecular hallmarks form the underpinnings of **facioscapulohumeral muscular dystrophy (FSHD).** This is a progressive disease characterized by muscle weakness. There is no cure for this disease.

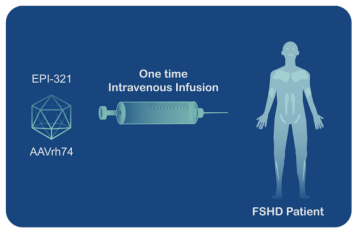


Figure 8. (B) Schematic showing proposal for a one-and-done strategy to treat FSHD. The therapeutic approach is uniquely possible with EPI-321, an epigenome editing technology that is designed to restore methylation to the affected locus and thereby suppress the stochastic and toxic DUX4 gene. Thus suppression will be achieved by epigenetic means, mitotically-stable, and long-lasting.

Conclusions and related posters

Epicrispr GEMS represents a powerful platform for therapeutic editing of the epigenome. Featuring compact component proteins, highly interchangeable, and programmable platform, GEMS offers the prospect of safe and precise epigenome editing both *ex vivo* and *in vivo*.

For more details please view:

Poster #71: Discovery and engineering of hypercompact transcriptional modulators for robust and durable target gene activation

Poster#80: EPI-321-A promising CRISPR epigenome engineering therapy for facioscapulohumeral muscular dystrophy (FSHD)

Poster #100: Combinatorial screening of transcription activation domains provides insights into biophysical properties of strong activators

Poster #117:Accelerating protein engineering with machine learning- A few-shot transfer learning approach to designing novel gene activators

Poster #215: Engineering of compact ribonucleoprotein complexes for epigenome editng