

Supporting Information

DNA-Templated Self-Assembly of Bradykinin into Bioactive Nanofibrils

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Content:

- ⇒ Supplementary methods;
- ⇒ LC-MS spectra from peptides;
- ⇒ Electrophoretic run from fragmented calf-thymus DNA;
- ⇒ Representative spectra from peptide and peptide/DNA samples in 1-anilino-8-naphthalene sulfonate (ANS);
- ⇒ Representative spectra in EtBr and SyBr safe;
- ⇒ Fluorescence assays in methyl green (major-groove binder);
- ⇒ Fluorescence in thioflavin-T solutions;
- ⇒ Additional TEM images;
- ⇒ Circular dichroism;
- ⇒ SAXS modeling;
- ⇒ Cytotoxicity assays.

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COMPLEMENTARY METHODS:

Circular dichroism (CD): CD experiments were conducted on a Jasco 810 spectropolarimeter. Peptide and peptide/DNA solutions were prepared in ultrapure water at a concentration of 0.5 mM peptide and 30 μ M DNA. The samples were left to equilibrate overnight before measurements, which were carried out using 1 mm pathlength cuvettes at room temperature. Data were collected in the range of 190-250 nm with 1 nm steps at a scanning rate of 100 nm/min. Each curve represented the average of 5 accumulations, and noise elimination was performed using FFT filters with a 5-point window. All data were background subtracted using water spectra.

Cytotoxicity assays: Cytotoxicity was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously reported.¹ Cells were cultivated under the same conditions as described in the main text and incubated with peptide or peptide/DNA at various concentrations for 24 hours. Afterward, the cells were incubated with 0.5 mg/ml MTT for 4 hours. The formazan crystals were dissolved by adding 100 μ L of DMSO and stirring for 10 minutes at 37 °C, protected from light. The resulting absorbances were measured at 570 nm using the SpectraMax M2 microplate spectrophotometer (Molecular Devices, San Jose, USA). The data were then plotted and analyzed using GraphPad Prism software (GraphPad Software, USA).

Liquid chromatography and mass spectra:

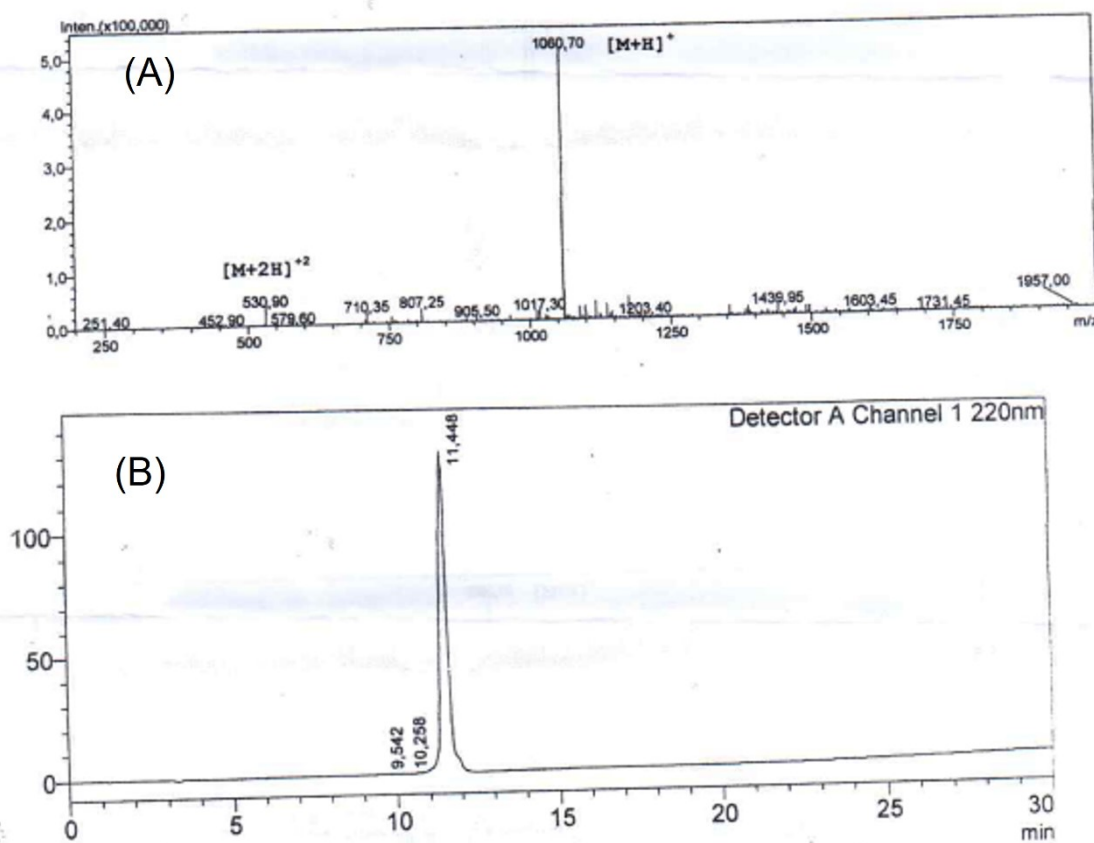


Figure S1. (A) Mass spectrum and (B) liquid chromatography spectrum from the bradykinin peptide. The purity level was measured at > 99%, whereas the molecular weight was identified at $M_w = 1060.7 [M+H]^+$.

Electrophoretic runs of the DNA batches used in the study:

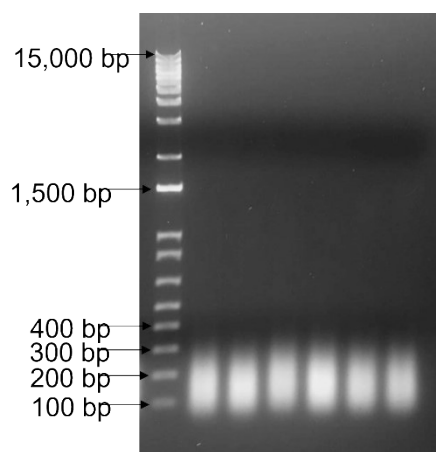


Figure S2. Electrophoretic run of calf-thymus DNA subjected to ultrasonication on a bioruptor. The resulting fragments have sizes around 150 base pairs.

Fluorescence spectra from BK and BK-DNA samples in the presence of 1-anilino-8-naphthalene sulfonate (ANS):

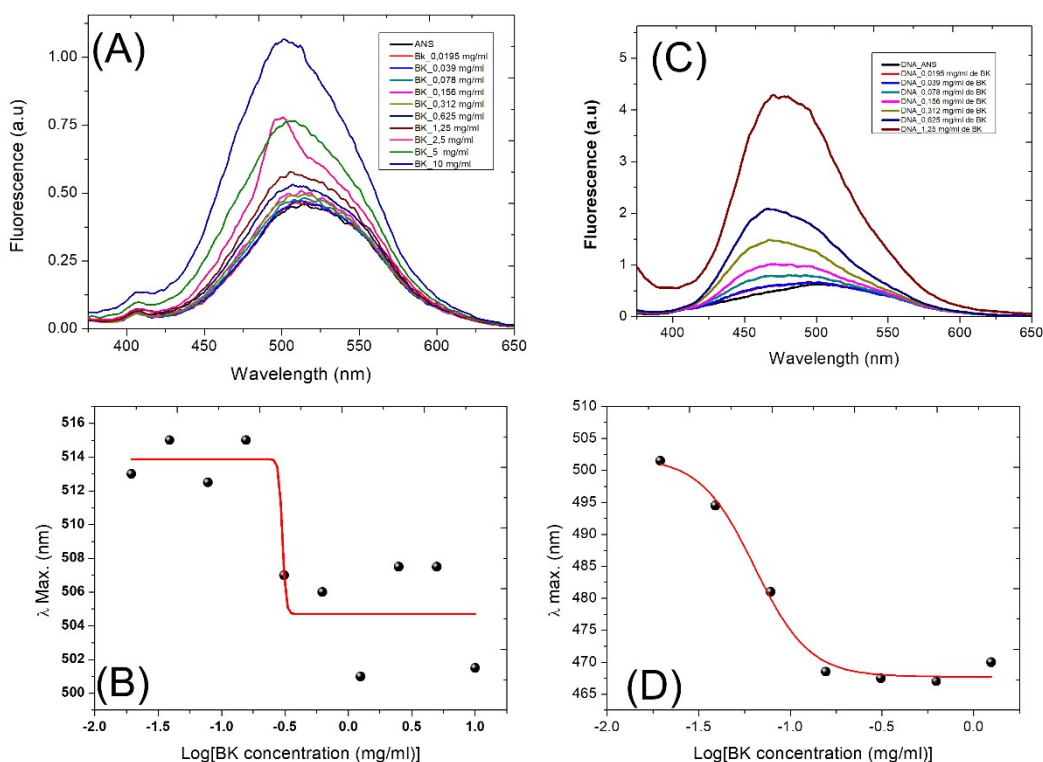


Figure S3. Fluorescence data from peptide and peptide//DNA solutions in the presence of ANS. Image (A) shows spectra from solutions containing BK at various concentrations, whereas (B) shows the wavelength of the maximum emission as a function of peptide concentration. In (C) and (D), the data correspond to samples containing BK mixed with 60 μ M DNA.

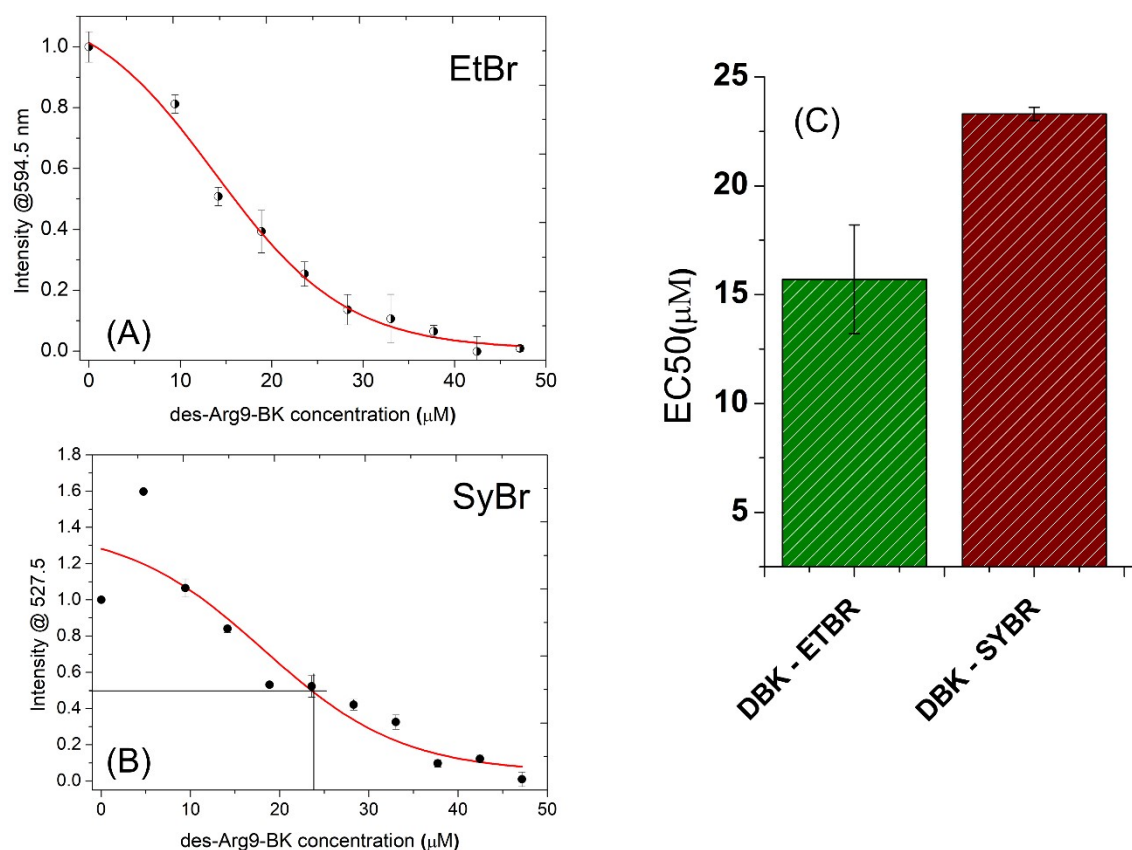


Figure S4. Dye displacement assays on DES-Arg9-BK titration series in 60 μM DNA complexed with the base-intercalating agent, EtBr (A), and the minor-groove binder, SyBr safe (B). The data are presented as the mean and standard deviation of triplicates, and the solid lines serve as a guide to the eye. (C) Comparison of the EC_{50} values found in the titration series.

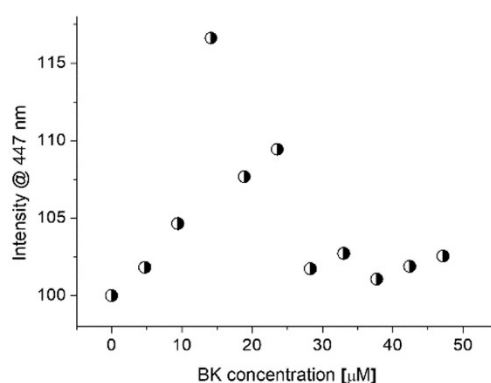


Figure S5. Fluorescence behavior of methyl green, a major-groove binder dye, in the presence of BK-DNA.

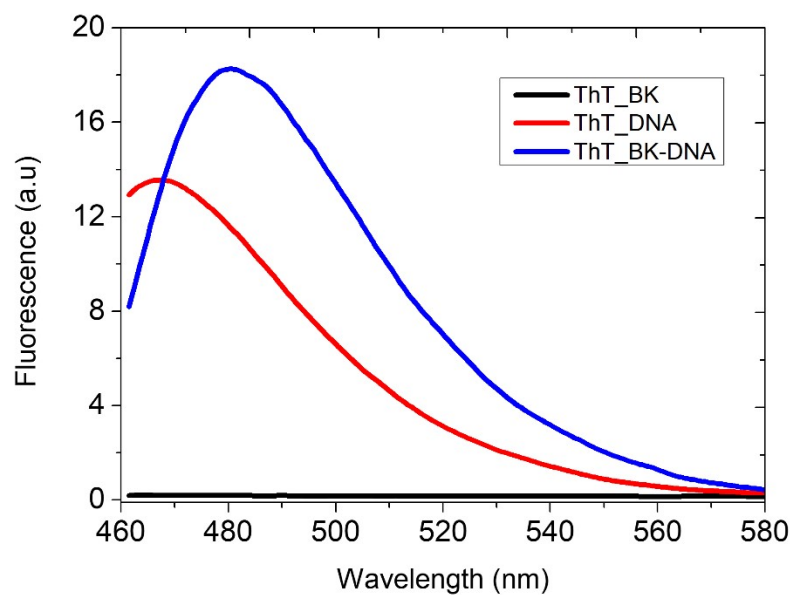


Figure S6. Fluorescence assays using Thioflavin-t as a probe. The observed increase in fluorescence intensity upon complexation provides strong evidence of an increase in order within the resulting aggregates.

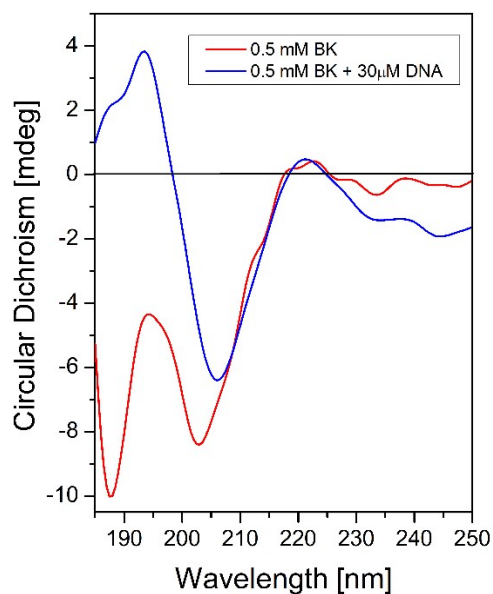


Figure S7. Circular dichroism spectra from BK and BK-DNA solutions. The peptide concentrations in the samples were 0.5 mM, and the complex was prepared by adding a small amount of DNA (30 μ M). The BK spectrum exhibits mixed signals with polyproline II² and β -sheet features, whereas the complex displays a distinct β -sheet spectrum. The experiments were conducted at room temperature, employing a cuvette with a 1 mm path length.

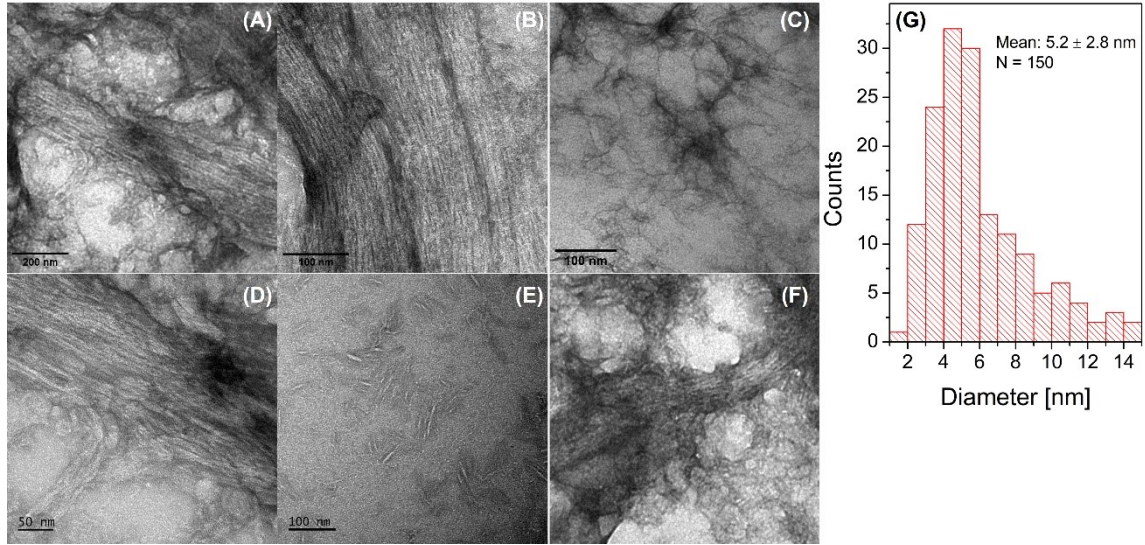


Figure S8. (A)-(F) Additional TEM images from BK-DNA nanofibers. (G) Histogram showing the frequency of fiber diameter across the images, based on over 150 measurements.

SAXS MODELING:

Generalized Gaussian chain model:

SAXS data from BK samples were fitted using the following expression:

$$I(q) \cong Bkg + \frac{I_0 \cdot U^{\frac{1}{2v}} \cdot \Gamma\left(\frac{1}{2v}\right) - \Gamma\left(\frac{1}{v}\right) - U^{\frac{1}{2v}} \cdot \Gamma\left(\frac{1}{2v}, U\right) + \Gamma\left(\frac{1}{v}, U\right)}{v \cdot U^{\frac{1}{v}}} \quad (S1)$$

where Bkg is an additive constant to describe a flat background. The second is the form factor of generalized Gaussian chains (gGc), largely used to describe SAXS data from polymer systems.³ Γ is the Gamma function and U is a modified variable given by:

$$U = (2\nu + 1)(2\nu + 2) \frac{q^2 \cdot R_g^2}{6} \quad (S2)$$

Although a certain complexity introduced by the gamma function, these model bears only two structural parameters, the radius of gyration R_g and the Flory exponent ν . R_g is associated to the characteristic size assumed by a chain in solution, being the root mean square of the distances to the center of mass of the volume occupied by the chain. The ν is the Flory excluded volume parameter, related to the polymer/solvent interactions. A value of $\nu \approx 0.5$ indicates that the chains are in theta solvents. A value of $\nu \approx 0.33$ is related to collapsed chains and $\nu \approx 0.6$ is associated to self-avoiding swollen chains.⁴

Porod cylinder form factor:

Data from DNA solutions are adequately fitted using the Porod form factor given by:^{3,4}

$$P_{cyl}(q, R, L) = \frac{2}{qL} (\Delta\eta R^2 L)^2 \times \left\{ Si_{\frac{\pi}{2}}(qL) \Lambda_1^2(qR) - \frac{2\Lambda_2(2qR) - \Phi(2qR)}{qL} - \frac{\sin(qL)}{(qL)^2} \right\} \quad (S3)$$

Where:

$$Si_{\frac{\pi}{2}}(x) = \left(Si(x) + \frac{\cos(x)}{x} + \frac{\sin(x)}{x^2} \right) \xrightarrow{x \rightarrow \infty} \frac{\pi}{2}$$

$$\Lambda_1(x) = \frac{2}{x} J_1(x)$$

$$\Lambda_2(x) = \frac{8}{x^2} J_2(x)$$

$$\Phi(x) = \frac{2}{x^2} [1 - \Lambda_1(x)]$$

Here R is the radius of the cylinder, whereas $\Delta\eta$ is the electron contrast variation between the solvent and the scatterers. L is the length of the cylinder. Since the lengths of the DNA fragments used here were out of the resolution limit of the technique, this parameter has been arbitrarily fixed at $L = 70$ nm.

Core-shell cylinder form factor:

Data from BK-DNA mixtures were satisfactorily fitted according to the cylindrical shell form factor, P_{CS} , is given by:³

$$P_{CS}(q) = Bkg + \left[\frac{2Si(qL)}{qL} - \left(\frac{\sin\left(\frac{qL}{2}\right)}{\frac{qL}{2}} \right) \right] \times \left(\frac{2J_1(qR_c)}{qR_c} (\eta_{core} - \eta_{shell}) R_c^2 L \pi + \frac{2J_1(q(R + \Delta R))}{q(R_c + \Delta R)} (\eta_{shell} - \eta_{solv}) (R_c + \Delta R)^2 \right) \quad (S4)$$

J_1 is the regular cylindrical first-order Bessel function and Si is the sine integral function. η_{shell} , η_{core} and η_{solv} are the electron densities of the shell, the cylinder core and solvent, respectively, which here were left arbitrarily free. L is the length of the cylinder, also behaving as scaling constant when $L \gg R$. Since TEM images showed that the fibrils have lengths much longer than their radii, we have arbitrarily fixed $L = 1000$ nm. R_c is the inner radius of the cylinder and ΔR is the thickness of the shell.

CYTOTOXICITY ASSAYS:

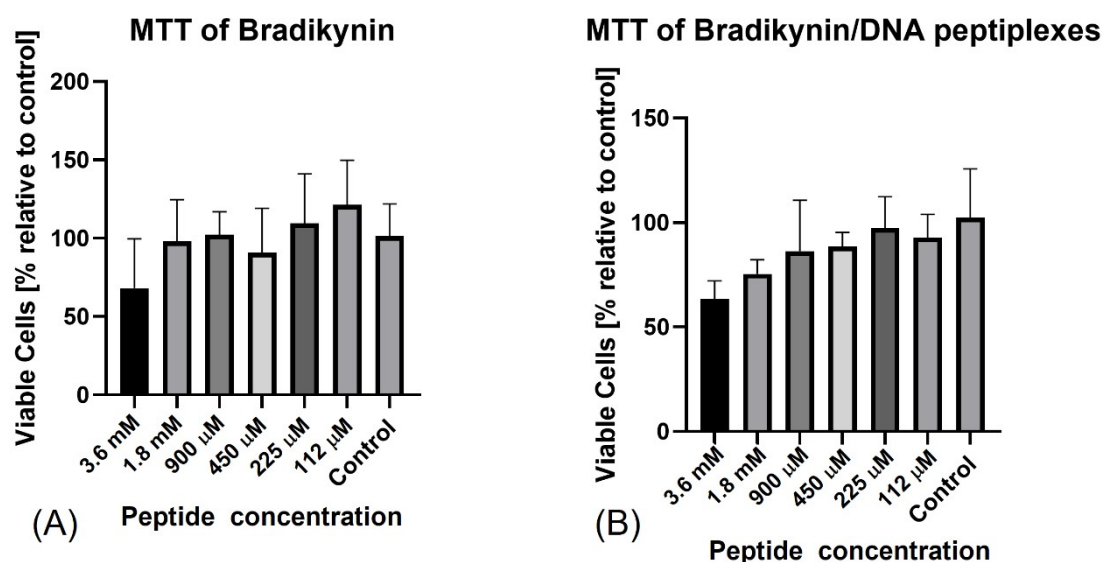


Figure S9. MTT assays conducted in HEK-293t cells incubated with (A) bradykinin or (B) BK-DNA complexes (charge ratio 2:1). ANOVA analyses, corrected by Bonferroni test, demonstrate no significant cytotoxicity compared to the control throughout the investigated range of peptide concentrations.

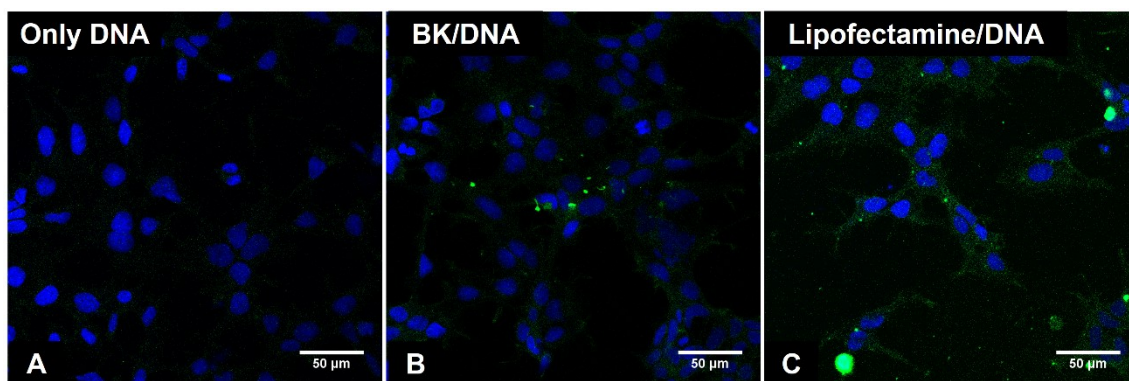


Figure S10. Comparison of green fluorescence levels in HEK293t cells incubated with (A) DNA alone, (B) BK/DNA complexes (2:1 molar ratio), and (C) lipofectamine/DNA complexes. Measurements of the intensity level in the green channel indicate that BK/DNA complexes enhance fluorescence by approximately 20%, while lipofectamine/DNA complexes result in an increase of approximately 60% compared to the DNA control.

References:

- (1) de Mello, L. R.; Porosk, L.; Lourenço, T. C.; Garcia, B. B. M.; Costa, C. A. R.; Han, S. W.; de Souza, J. S.; Langel, Ü.; da Silva, E. R. Amyloid-like Self-Assembly of a Hydrophobic Cell-Penetrating Peptide and Its Use as a Carrier for Nucleic Acids. *ACS Appl. Bio Mater.* **2021**, 4 (8), 6404–6416.
- (2) Nordén, B.; Rodger, A.; Dafforn, T.; Royal Society of Chemistry (Great Britain). *Linear Dichroism and Circular Dichroism : A Textbook on Polarized-Light Spectroscopy*; Royal Society of Chemistry: Cambridge, 2010.
- (3) Pedersen, J. S. Analysis of Small-Angle Scattering Data from Colloids and Polymer Solutions: Modeling and Least-Squares Fitting. *Adv. Colloid Interface Sci.* **1997**, 70 (0), 171–210.
- (4) Zemb, T.; Lindner, P. *Neutrons, X-Rays and Light : Scattering Methods Applied to Soft Condensed Matter*, 1st ed.; Elsevier: Amsterdam ; Boston, 2002.