

## Translocation of a $\beta$ -Peptide Across Cell Membranes

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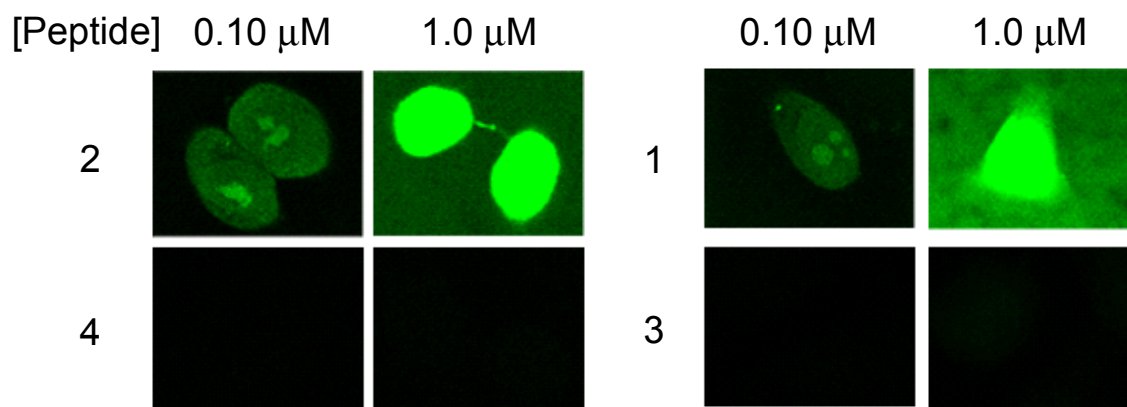
### Supporting Information

#### Synthesis and purification of **1**.

$\beta$ -Peptides such as **1** can be conveniently prepared with conventional automated solid-phase synthesis equipment, although the coupling and deprotection steps require more time than do analogous steps in  $\alpha$ -peptide synthesis. Preparation of **1** was challenging because 6 of the 11 residues are  $\beta^3$ -homoarginine ( $\beta^3$ hArg). The protected (*S*)- $\beta^3$ hArg monomer was synthesized by Arndt-Eistert homologation,<sup>14</sup> as modified by Müller et al.,<sup>15</sup> starting from Fmoc-L-Arg with 2,2,5,7,8-pentamethyl-chroman-6-sulfonyl (Pmc) protection of the sidechain guanidino group. Conventional purification provided Fmoc-(*S*)- $\beta^3$ hArg(Pmc) contaminated with small amounts ( $\leq 1\%$ ) of Fmoc-L-Arg(Pmc). It was impossible to detect this impurity by NMR or TLC; use of impure Fmoc-(*S*)- $\beta^3$ hArg(Pmc) in syntheses of **1** led to  $\alpha$ -Arg-containing oligomer contaminants that could be detected by mass spectrometry but were impossible to separate by HPLC. This problem was circumvented by developing an HPLC assay to monitor Fmoc-L-Arg(Pmc) impurity levels. Only Fmoc-(*S*)- $\beta^3$ hArg(Pmc) without detectable impurity ( $< 0.05\%$ ), obtained via repeated column chromatography, was used for oligomer synthesis.

**HPLC assay for Fmoc- $\beta^3$ -homoarginine(Pmc) purity:** Alltech C-18 analytical column (Waters Spherisorb ODS-2 5U), 9:1 solvent A:solvent B to 2:8 solvent A:solvent B over 70 min, followed by a 10 min flush with 5:95 solvent A:solvent B and a 10 min flush with 9:1 solvent A:solvent B, where solvent A = 100 mM aqueous NaOAc (pH 6.5) containing 10% (v/v) CH<sub>3</sub>CN, and solvent B = CH<sub>3</sub>CN containing 10% (v/v) water. Under these conditions Fmoc- $\beta^3$ HArg(Pmc) and Fmoc-L-Arg(Pmc) elute between 40 and 50 min with retention times that differ by ca. 4 min.

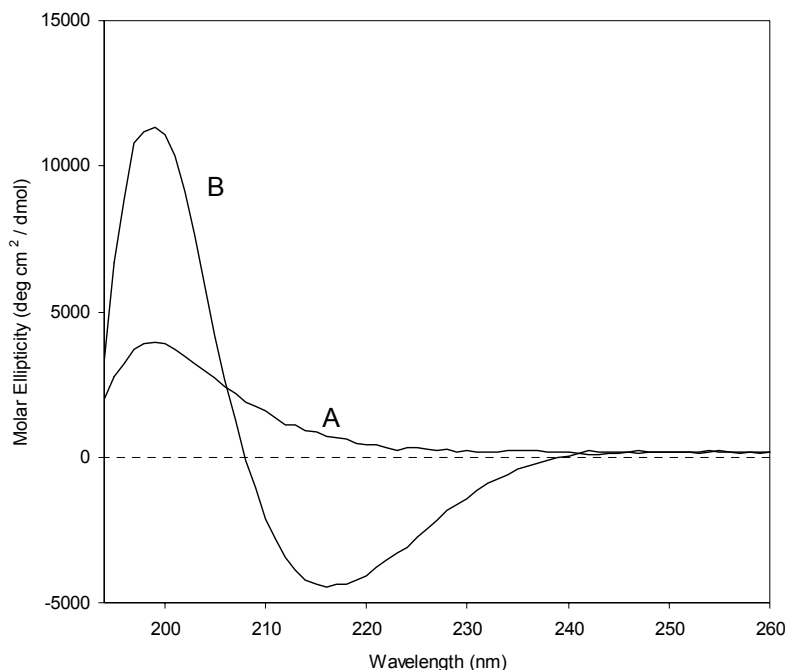
#### Additional confocal microscopy image.



**Figure S1.** Confocal microscopy images of HeLa cells incubated for 10 min at 37 °C with a solution 0.10  $\mu$ M fluorescein-labeled peptides **1**, **2**, **3**, or **4**, washed, and fixed as for Figure 1 in the text. The dark panels show that peptides **3** and **4** are not internalized, even at 1.0  $\mu$ M. The microscope settings were identical for each peptide and dose. Bar: 10  $\mu$ m.

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**Figure S2.** Circular dichroism data obtained on an Aviv 202SF instrument with 1-mm pathlength cells at 25 °C for a solution of 0.12 mM  $\beta$ -peptide **5** in water (A) and methanol (B); data are shown as mean residue ellipticity. Several studies suggest that the random coil state of  $\beta$ -peptides displays no characteristic CD maxima or minima in the far-UV region,<sup>A</sup> but **5** in water displays a maximum at 199 nm. This CD spectrum is reminiscent of the signature for the 10/12-helix (defined by alternating 10-membered ring  $i \rightarrow i-1$  C=O--HN hydrogen bonds and 12-membered ring  $i \rightarrow i+3$  C=O--HN hydrogen bonds), which has a maximum at 205 nm.<sup>B</sup> The intensity of the CD maximum of **5** in aqueous solution, however, is quite low relative to 10/12-helix precedents in methanol,<sup>B</sup> which suggests that **5** is largely disordered in water. In organic solvents, many  $\beta$ -peptides constructed from  $\beta^3$ -residues adopt a 14-helical conformation (multiple 14-membered ring  $i \rightarrow i-2$  C=O--HN hydrogen bonds).<sup>C</sup> The stability of this conformation among  $\beta$ -peptides comprised of  $\beta^3$ -residues is much higher in methanol than in water.<sup>A(i),D</sup> In contrast to Tat 47-60, which remains unstructured in methanol,<sup>6b</sup>  $\beta$ -peptide **5** displays the characteristic 14-helical CD signature, with a minimum at 216 nm and a maximum at 199 nm. Thus,  $\beta$ -peptide **1** has an intrinsically higher propensity than does  $\alpha$ -peptide **2** to adopt an ordered conformation in non-aqueous environments.

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<sup>B</sup> Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Matthews, J. L.; Schreiber, J. V. *Helv. Chim. Acta* 1998, *81*, 932. Seebach, D.; Gademann, K.; Schreiber, J. V.; Matthews, J. L.; Hintermann, T.; Jaun, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* 1997, *80*, 2033.

<sup>C</sup> Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* 1996, *79*, 913. Seebach, D.; Ciceri, P. E.; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta* 1996, *79*, 2043.

<sup>D</sup> Abele, S.; Guichard, G.; Seebach, D. *Helv. Chim. Acta* 1998, *81*, 2141. Appella, D. H.; Joseph J. Barchi, J.; Durell, S. R.; Gellman, S. H. *J. Am. Chem. Soc.* 1999, *121*, 2309.