

# Assessing Branched Amphiphilic Peptide Capsules (BAPC) Delivery of Short Interfering RNA (siRNA) to Mammalian Cells

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## Objectives

**Primary:** To evaluate knockdown of target protein following antisense siRNA delivery to GFP-CHO cells with BAPC versus current commercial transfection reagents. **Secondary:** To assess cellular viability through cryopreservation following BAPC treatment.

## Experimental design

**Transfection efficiency.** CHO-GFP cells were transfected with the following double-stranded RNA complexes: 1) siRNA237 (5' gcagcagcagcuucucaag 3' and 5' cuugaagaagucgugcugc 3') and 2) mission siRNA universal negative control (Millipore). GFP-CHO and CHO (negative control) cells were resuspended in maintenance media and plated at  $1 \times 10^5$  cells/well in 24-well plates. Treatments were initiated after cells reached  $\geq 75\%$  confluence. For comparison of transfection reagents, we used the following reagents combined with 25 nM siRNA:

- Lipofectamine 3000 (ThermoFisher) per manufacturer's instructions
- TransIt-X2 (Mirus Bio) per manufacturer's instructions
- BAPC with fluorescent tracker (633 nm) in 3 variants: 25 nm mean particle size at concentrations of 0.01, 0.1, or 1  $\mu\text{M}$ , pre-incubated with siRNA for 30 min

The transfected cells were incubated at 37°C with 5% CO<sub>2</sub> in a humidified incubator for 48 h. The suppression of each target transcript were evaluated using fluorescence microscopy and the Guava® easyCyte Flow Cytometer System (Millipore) to detect green (GFP) fluorescence.

Negative control siRNA treatments were used to assess off-target effects of reagents on cell function, whereas siRNA237 were used to assess target knockdown. Cell viability were assessed using rezasurin method (Riss et al., 2004).

**Viability after cryopreservation.** The 3 BAPC variants from experiment 1 were assessed for potential carryover effects on cryopreserved cells. Positive and negative control treatments and BAPC treatments, complexed with negative control siRNA and siRNA237, were applied to GFP-CHO cells in triplicate in 6-well culture plates. After 48 h, cells were cryopreserved in FBS with 10% DMSO and maintained in liquid nitrogen for one month. After storage, each vial was thawed and cells were plated back on 6-well plates. 48 h later, metabolic activity (accounting for both expanded cell populations and metabolic activity per cell) were assessed by rezasurin metabolism.

Results:

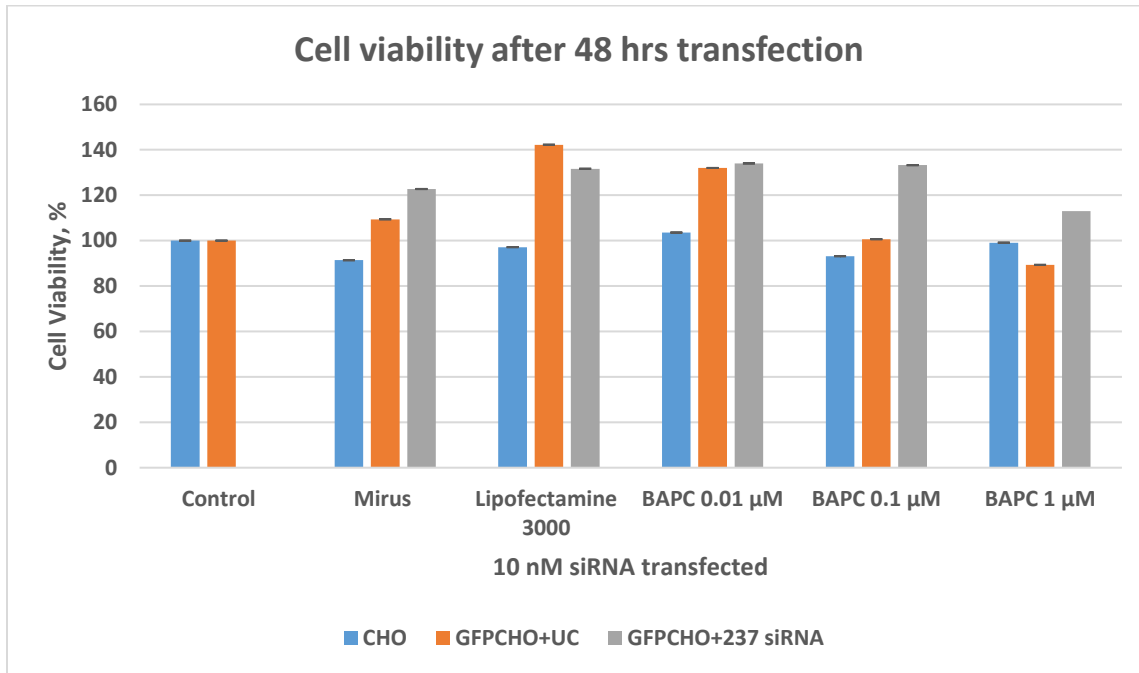


Figure 2A.

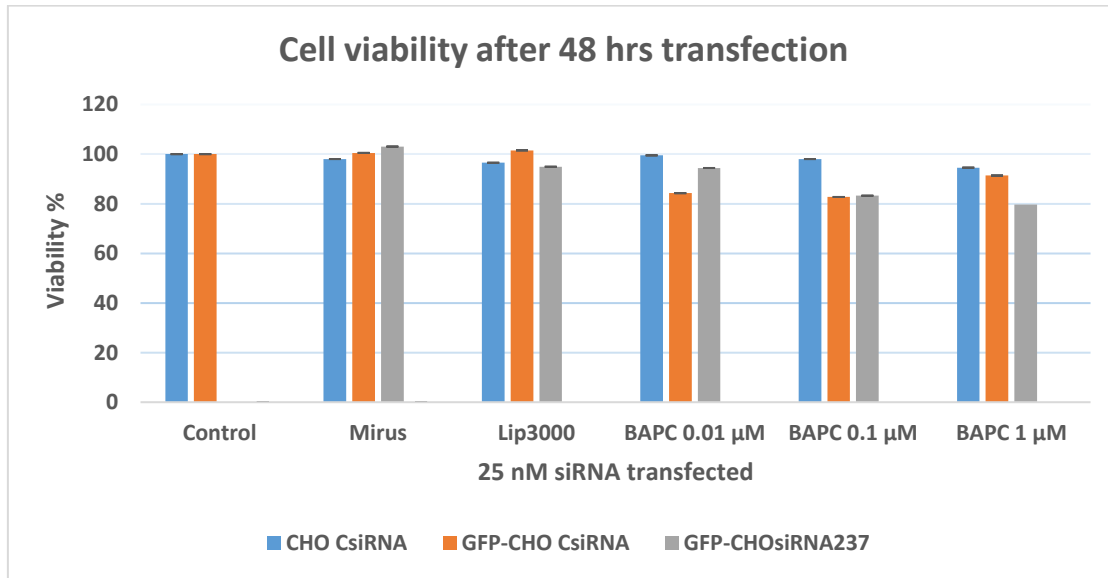


Figure 2B.

Figure 2 A and B. **Cell viability after transfection.** BAPC were dissolved per BAPtofect instructions using pure water and  $\text{CaCl}_2$ . 10 nM siRNA and BAPC, were incubated for 30 min, and then added to cells. Medium was changed after 4 h of transfection and cells were incubated for an additional 44 h. Cells were then treated with 10% resorufin and cell viability measured after 4 h incubation. 2B. Cells were transfected with 25 nM siRNA as described for Figure 2A and no medium were changed after 4 hours of

transfection. Cell viability were assessed with 10% resorufin and cell viability measured after 4 h incubation.

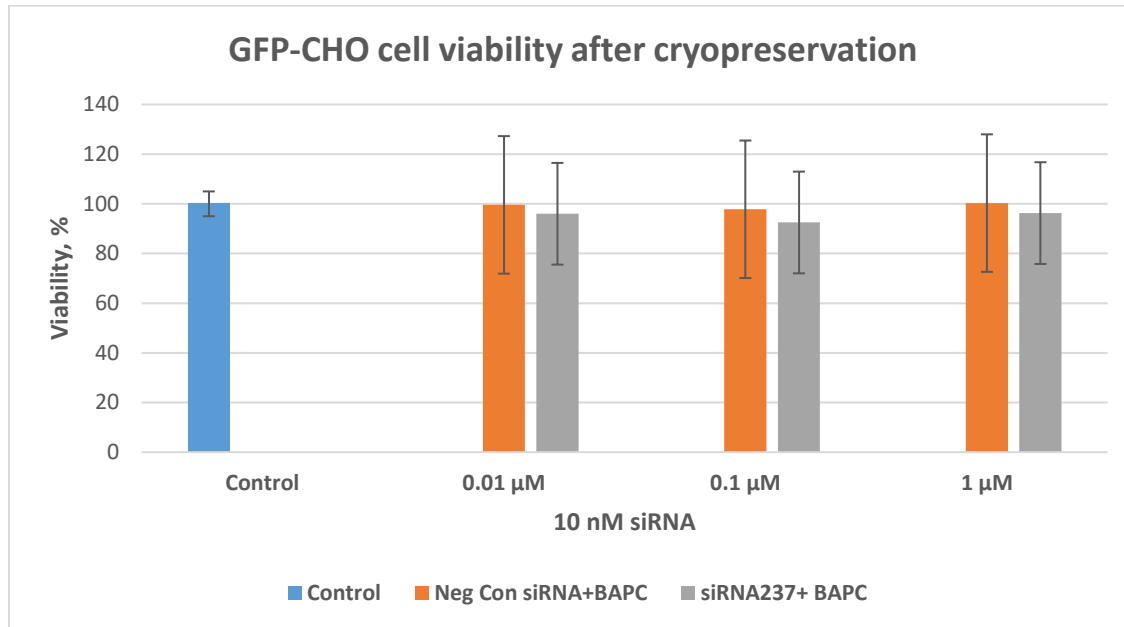


Figure 3.

**Figure 3. Viability after cryopreservation.** GFP-CHO cells transfected using different concentration of BAPC (0.01, 0.1 and 1 μM) with 10 nM negative control siRNA and siRNA 237 for 48 hours. After 48 h, cells were cryopreserved in FBS with 10% DMSO and maintained in liquid nitrogen for one month. After storage, each vial was thawed and cells plated back on 6-well plates. 48 h later, metabolic activity (accounting for both expanded cell populations and metabolic activity per cell) were assessed by rezasurin metabolism. Total of 6 replicates per treatment.

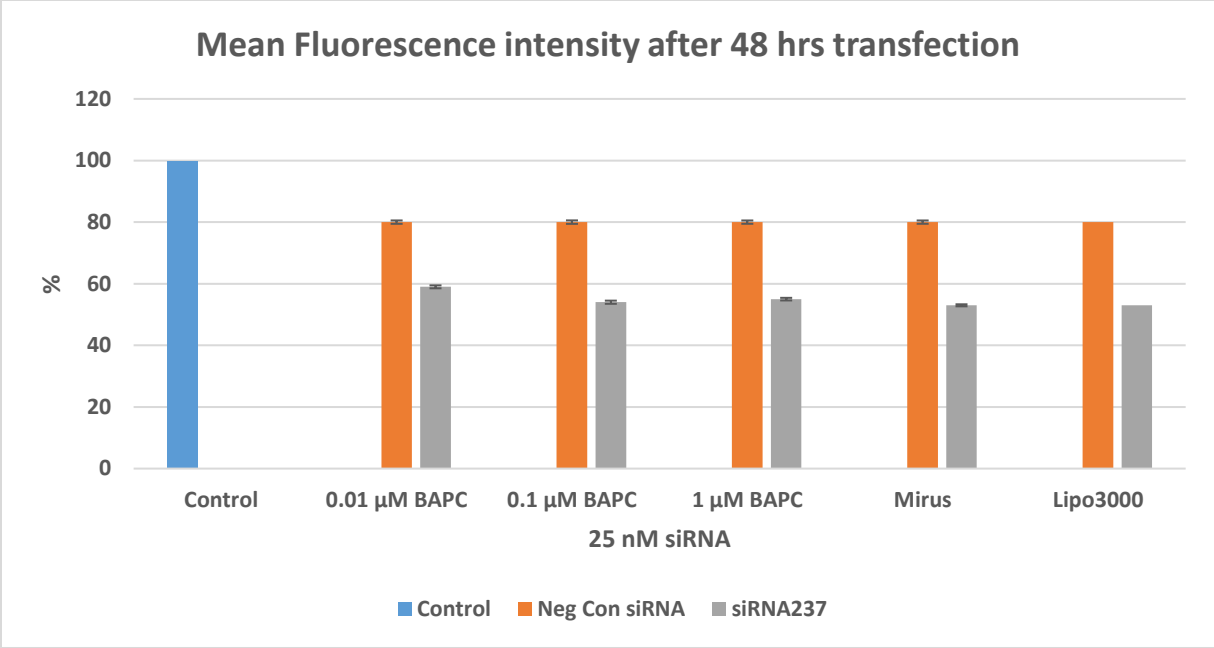


Figure 4A.

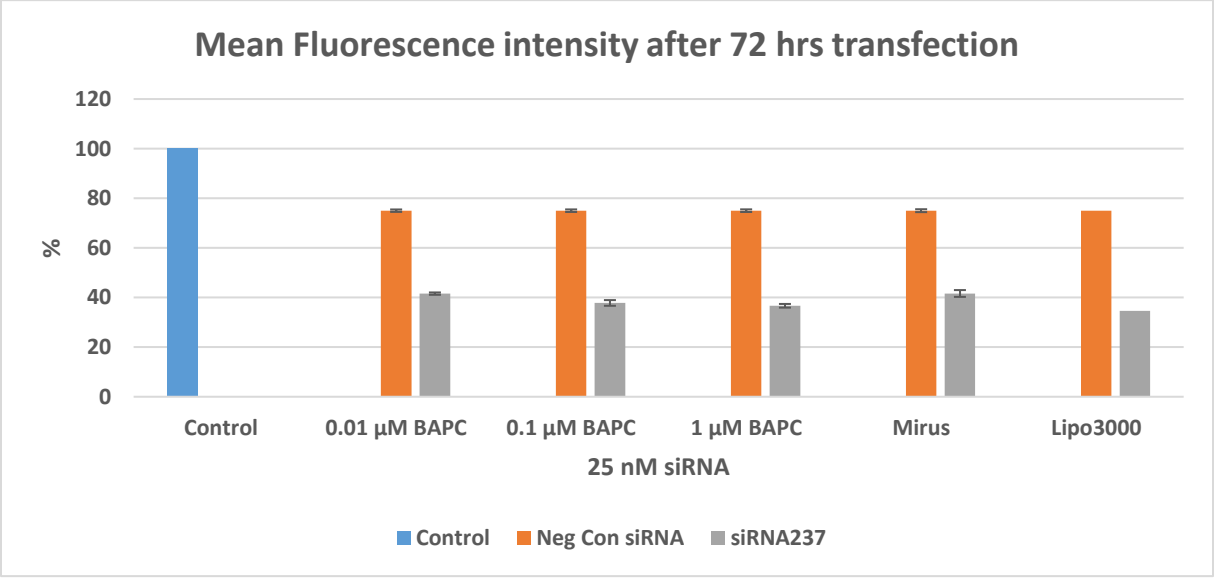


Figure 4B.

**Figure 4 A and B. Transfection efficiency.** GFP-CHO cell fluorescence 48-72 hrs. after transfection with negative control siRNA and siRNA237 using three different transfection reagents. GFP-CHO cells were transfected with 25 nM negative control and target siRNA 237. Cell medium has not been replaced after 4 hrs. transfection. There are 8 replicates per treatment.

**Conclusion:** BAPC at lower concentrations (0.01, 0.1 and 1 μM) does not reduce cell viability. BAPC at three different concentrations assessed knockdown siRNA 237 about 50 % after 48-72 hours. There is no

difference between BAPC and other transfection agents such as Mirus or Lipofectamine 3000. There is no dose effect between 0.01 – 1  $\mu$ M BAPC. BAPC did not affect cell viability after one-month cryopreservation.