

# Allosteric responsive DNA nanocages for specific miR21 sequestering in cancer cells



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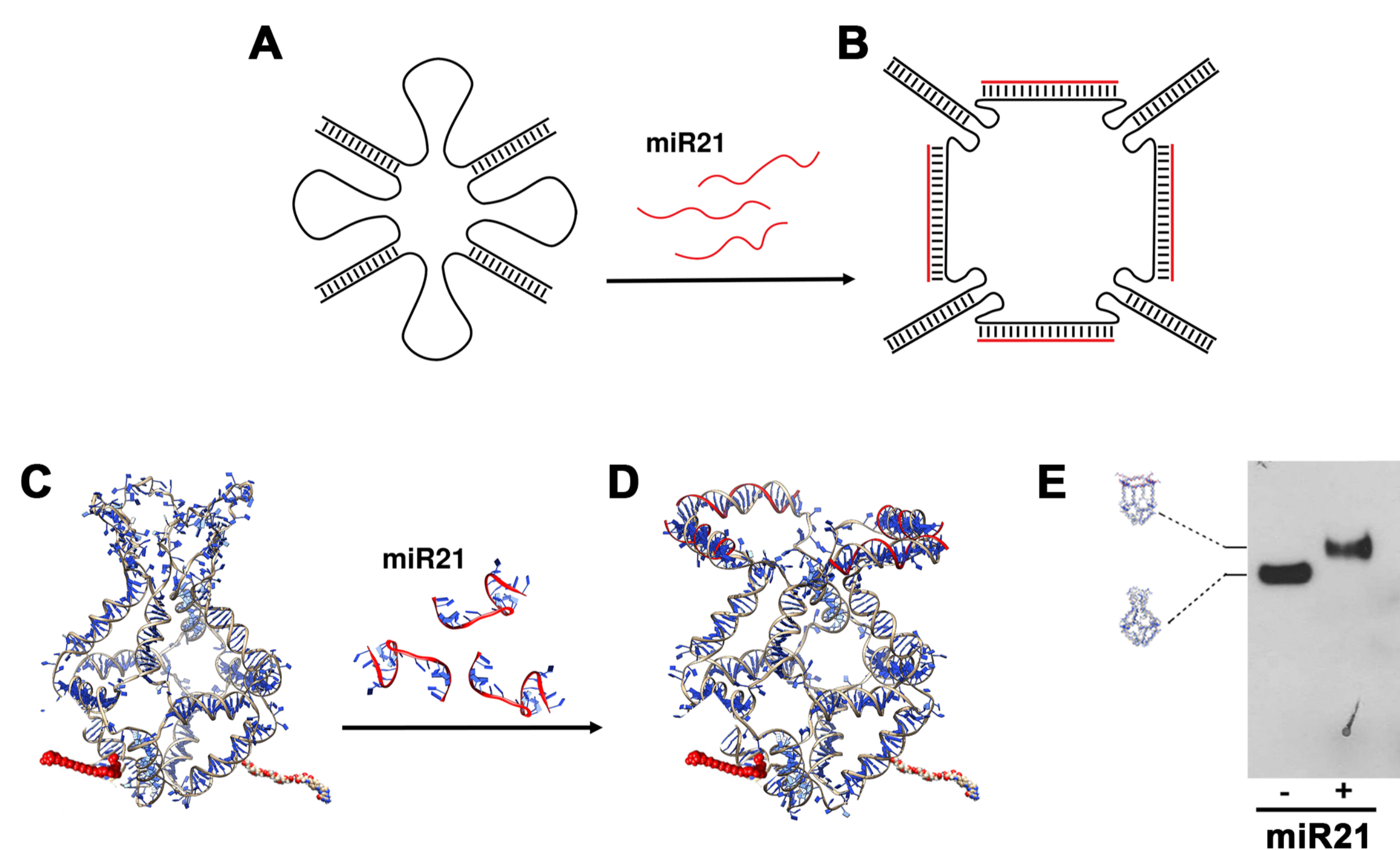
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Due to their biocompatible, nontoxic, and stable properties, DNA-based nanostructures have been proposed in various biomedical applications, such as drug delivery, cellular biosensing, and, more recently, miRNA-based anticancer therapy. MicroRNAs play an important role in cancer and, among them, miR21 is found to be aberrantly up-regulated in different tumors. Dysregulation of miR21 expression is related with the proliferation, apoptosis and migration of cancer cells.

Here, DNA nanocages were functionalized with folate molecules and utilized as scaffolds to engineer 4 sequestering units with a miR21 complementary sequence for obtaining biocompatible Fol-miR21-NCs nontoxic nanostructures able to selectively recognize folate receptor alpha-overexpressing cancer cells and sequester the oncogenic miR21. The complementary oligonucleotide act as an allosteric remodeler, inducing a conformational change to the nanocage, which displays a stable opened form larger than the closed form, as evidenced by electrophoretic analysis. Stability of Fol-miR21-NCs was investigated in biological fluids and in cells. RT-qPCR assays showed that Fol-miR21-NCs reduce the miR21 expression up to 80% in cancer cells in the first two days of treatment. Functional assays, such as up-regulation of miR21 tumor suppressor targets (i.e. PTEN and Pcd4), and reduction in cancer cell migration will be presented. These DNA nanostructures are easily modifiable through the insertion of specific sequences complementary to different miRNAs and can represent a versatile tool for multiple sequestering of overexpressed oncomiRs in cancer cells.

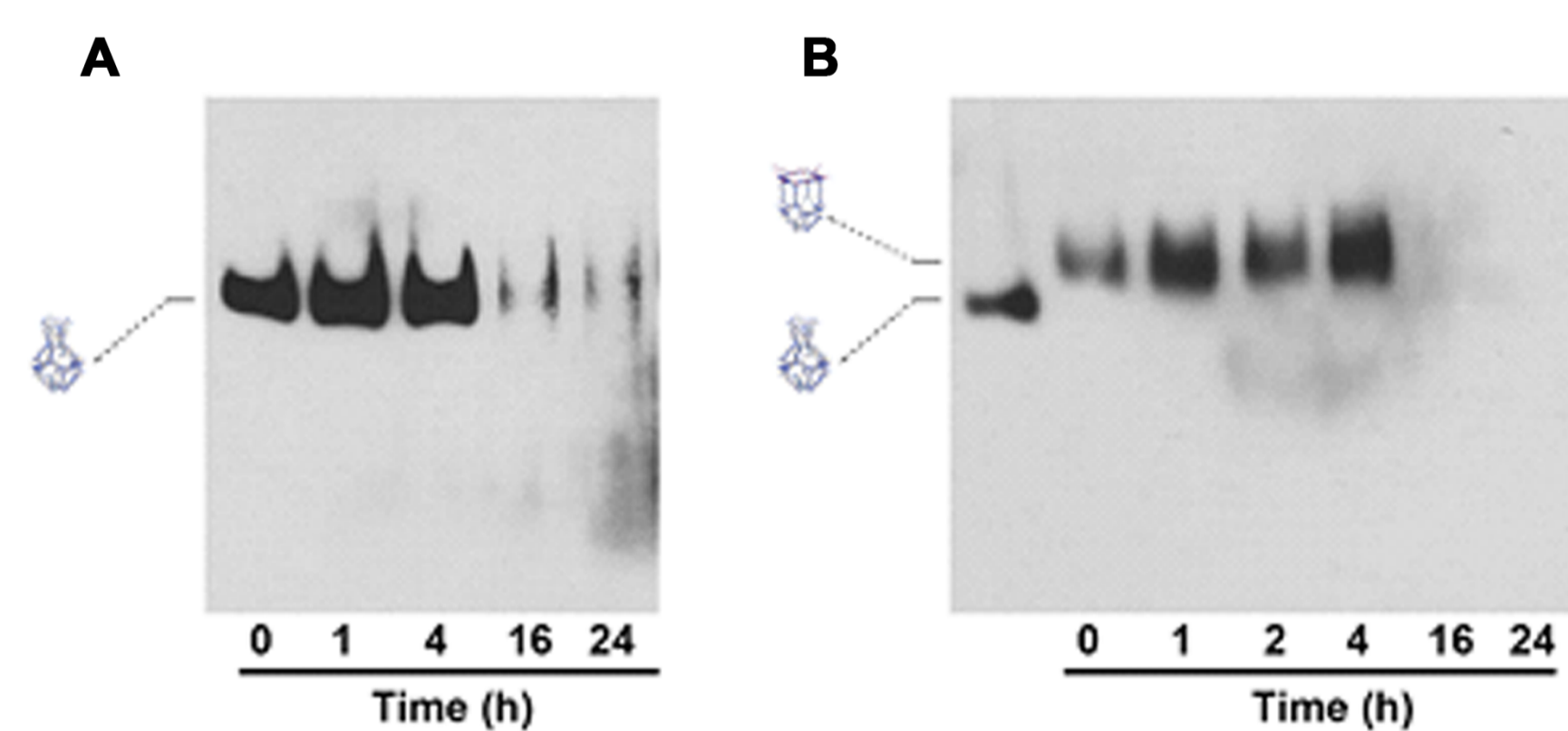
## Fol-miR21-NCs in silico and in vitro characterization

### 1. Binding of miR21 induces a conformational change in Fol-miR21-NCs toward an opened conformation



Schematic and atomistic representation of Fol-miR21-NC representation of the two states of Fol-miR21-NCs, showing the conformational change occurring in the Fol-miR21-NC after binding to miR21. Four miR sequestering units were introduced in one face of a truncated octahedral DNA nanocage. The four sequestering units have 21 nucleotides DNA sequence, complementary to miR21. (A) Schematic top view of the four sequestering units in the unbound state. (B) Schematic top view highlighting the bound state of Fol-NC upon interaction with miR21 molecules. (C, D) Three-dimensional representation of the two states, showing the conformational change occurring in the Fol-miR21-NC after binding to miR21. (E) Fol-miR21-NC in vitro opening reaction. DNA blot of Fol-miR21-NC electrophoretic mobility in the closed and opened conformational state before (-) and after (+) incubation with 10x molar excess of miR21.

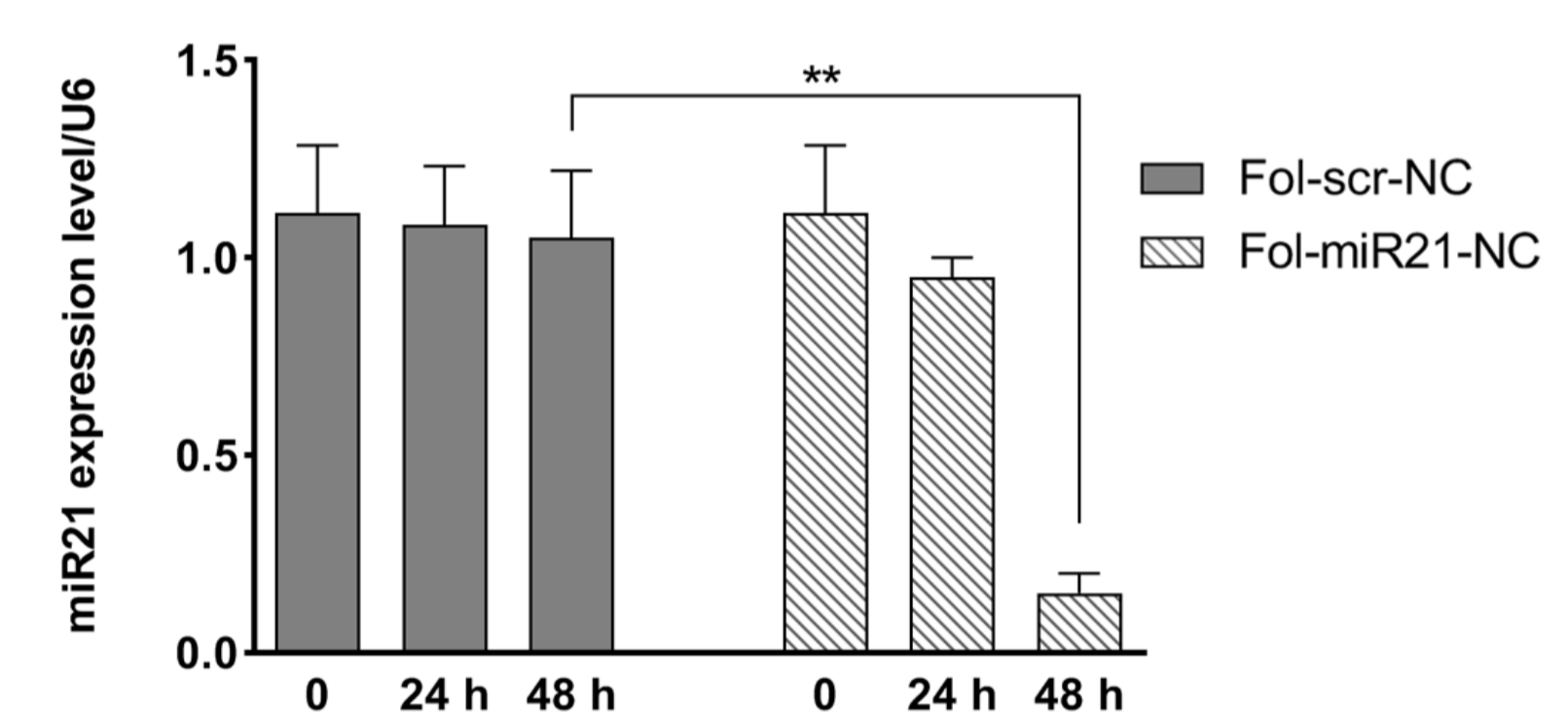
### 2. Closed Fol-miR21-NCs are more stable than the opened conformation in biological fluids



DNA blot analysis of (A) closed and (B) opened Fol-miR21-NCs incubated with 10% FBS for different times and detected by using Streptavidin-HRP. For testing the stability of the opened state, DNA nanocages were incubated in the presence of 10x molar excess of miR21 for 30 minutes to switch their conformation. Closed and opened structures were incubated with 10% FBS for different time intervals at 37°C, digested with Proteinase K (100 µg/mL), run in 5% polyacrylamide gel and blotted to examine their structural integrity.

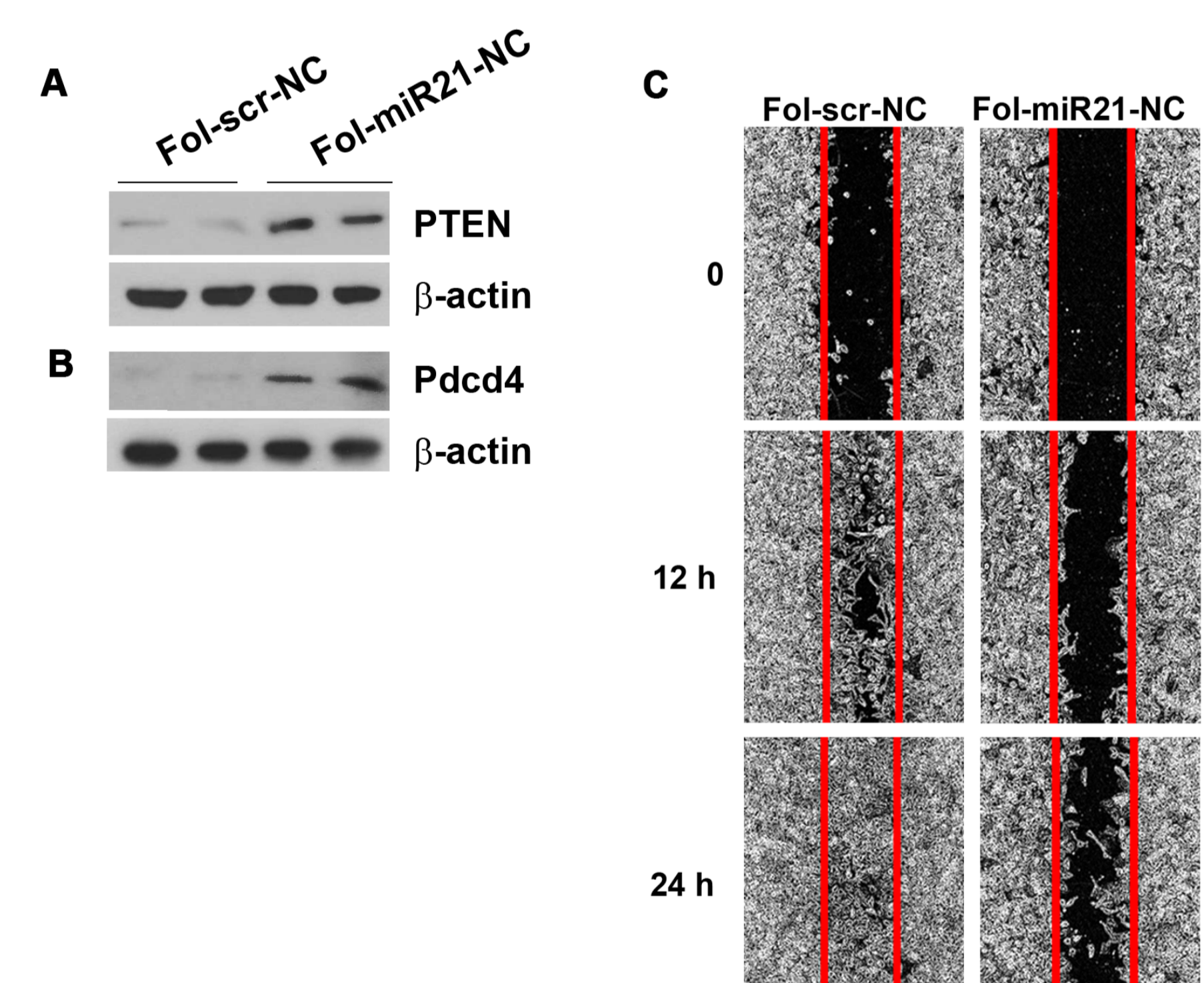
## Fol-miR21-NCs functional assays

### 3. Fol-miR21-NCs sequester miR21 and reduce its expression in HeLa cancer cells.



MiR21 silencing activity of Fol-miR21-NCs compared to Fol-scr-NCs. qPCR analysis of miR21 expression level is reported for HeLa cells incubated with Fol-NCs for different time intervals.

### 4. MiR21 silencing causes up-regulation of miR21 tumor suppressor targets and reduction of cell migration in HeLa cancer cells.



Fol-miR21-NCs functional assays on HeLa cells. Western blots of (A) PTEN and (B) Pcd4 in HeLa cells lysates, treated for 48h and 72h, respectively. (C) In vitro wound assay on cell migration across the scratched area was monitored for 24 h. Fol-scr-NCs were used as negative controls.

### Conclusions:

- Fol-miR21-NCs selectively recognize folate receptor alpha-overexpressing cancer cells and sequester the oncogenic miR21;
- Fol-miR21-NCs reduce the miR21 expression up to 80% in cancer cells after 48 h of treatment as shown by RT-qPCR assays;
- miR21 sequestering by Fol-miR21-NCs leads to up-regulation of PTEN and Pcd4 proteins and reduction of cell migration.

### References:

1. G. Vindigni, S. Raniolo, A. Ottaviani, M. Falconi, O. Franch, B. R. Knudsen, A. Desideri and S. Biocca. Receptor-Mediated Entry of Pristine Octahedral DNA Nanocages in Mammalian Cells. ACS Nano 2016
2. S. Raniolo, G. Vindigni, A. Ottaviani, V. Unida, F. Iacovelli, A. Manetto, L. Stella, A. Desideri and S. Biocca, Selective targeting and degradation of doxorubicin-loaded folate-functionalized DNA nanocages. Nanomedicine 2018.
3. S. Raniolo, G. Vindigni, V. Unida, A. Ottaviani, E. Romano, A. Desideri and S. Biocca. Entry, fate and degradation of DNA nanocages in mammalian cells: a matter of receptors. Nanoscale, 2018.
4. S. Raniolo, F. Iacovelli, V. Unida, A. Desideri and S. Biocca. In Silico and In Cell Analysis of Openable DNA Nanocages for miRNA Silencing. Int J Mol Sci., 2019