AD for targeted siRNA delivery

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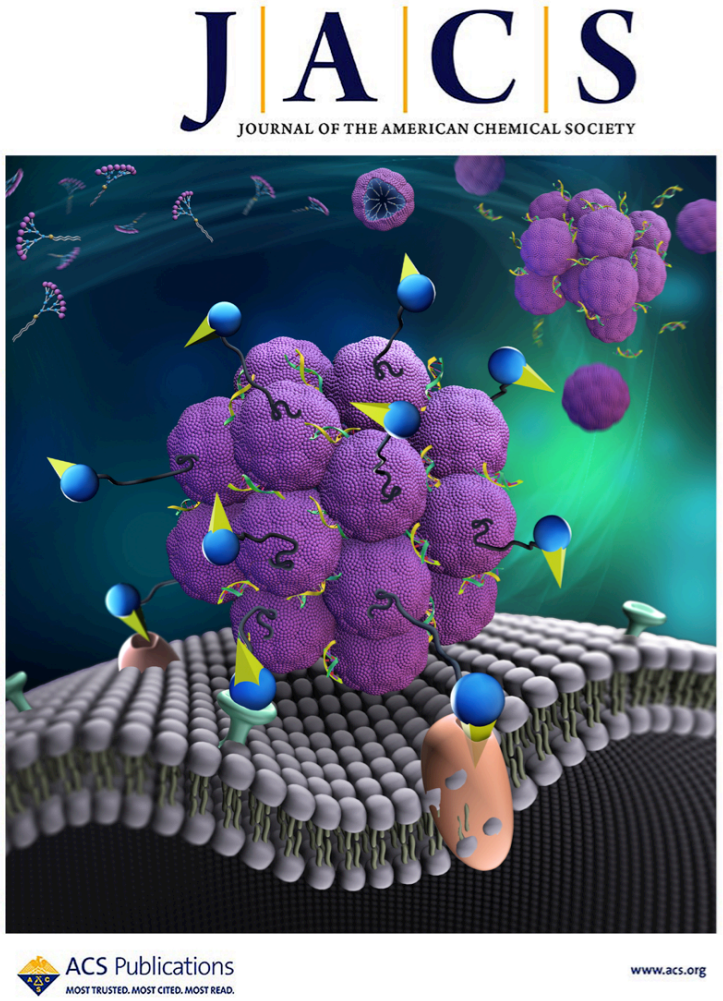
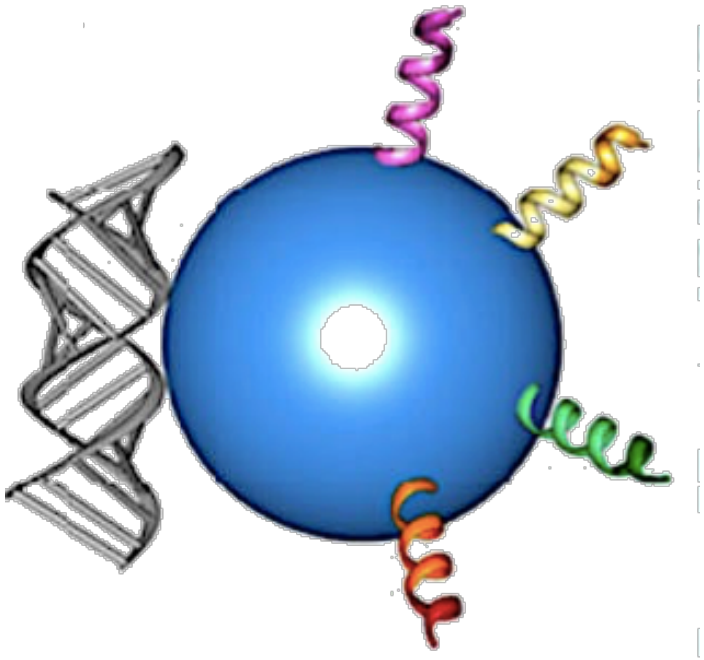
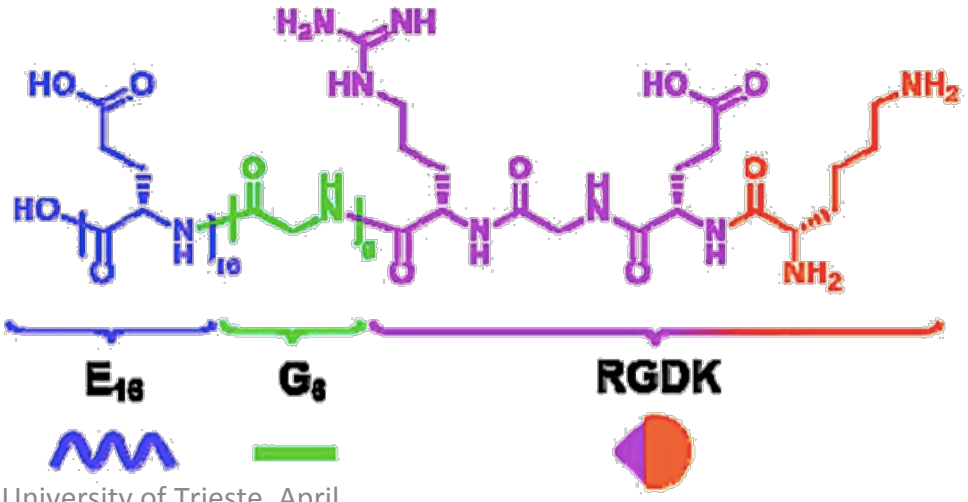
- Further improvement of **AD**-mediated siRNA delivery
  - Active targeting ability for specific cancer cell delivery within the lesion
- Dual targeting warhead RGDK peptide
  - Targets tumor endothelium by binding to $\alpha_v\beta_3$ integrin
  - Binds the neuropilin-1 (Nrp-1) receptor promoting cancer cell penetration and uptake
- The new **AD**/RGDK system is 10x more potent than the **AD** precursor



siRNA/**AD**/RGDK design and optimization





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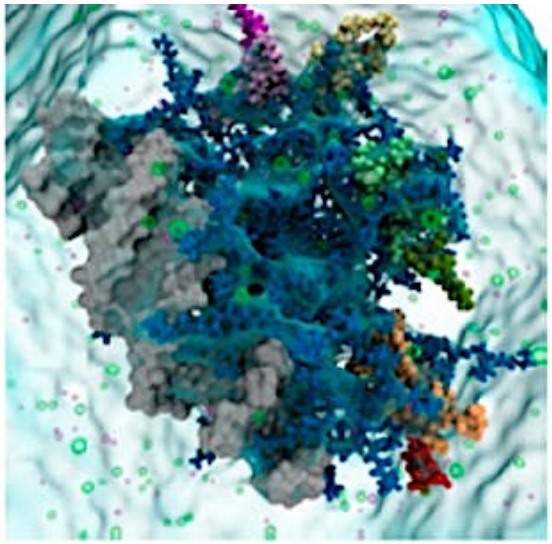
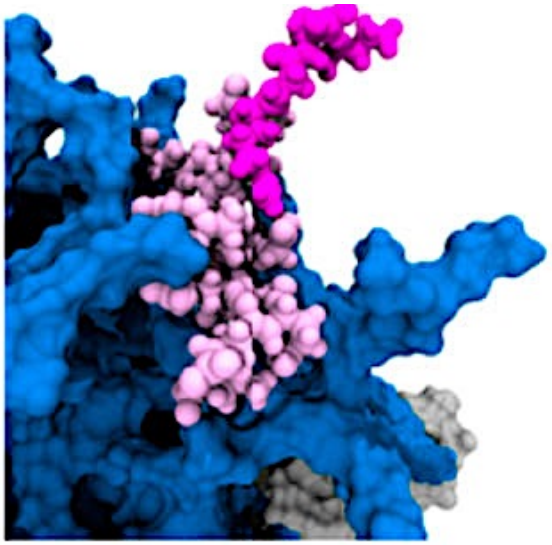
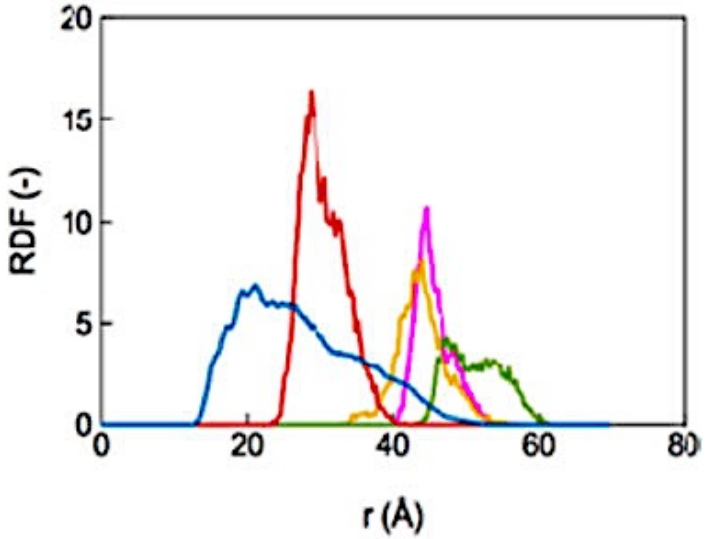
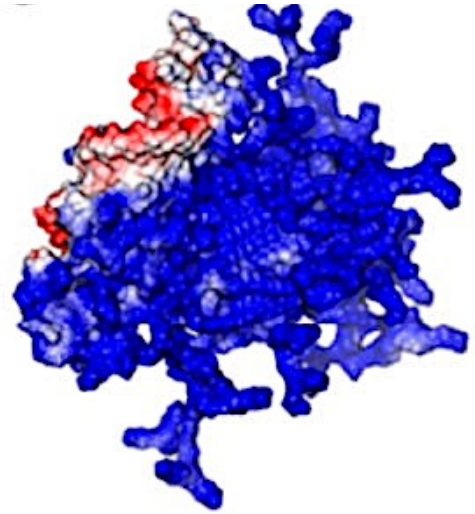
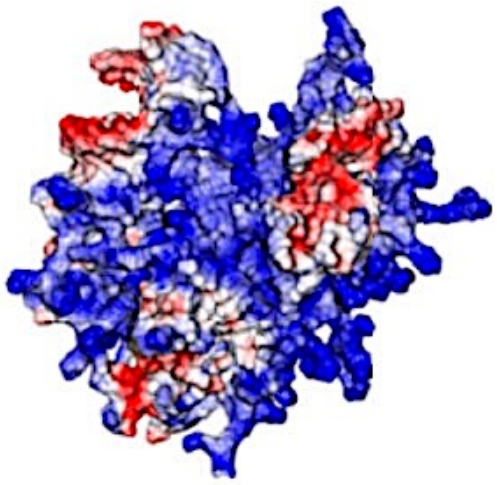
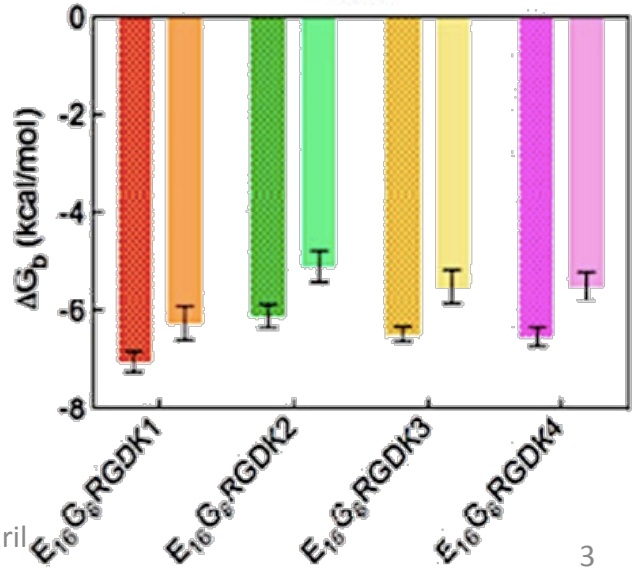




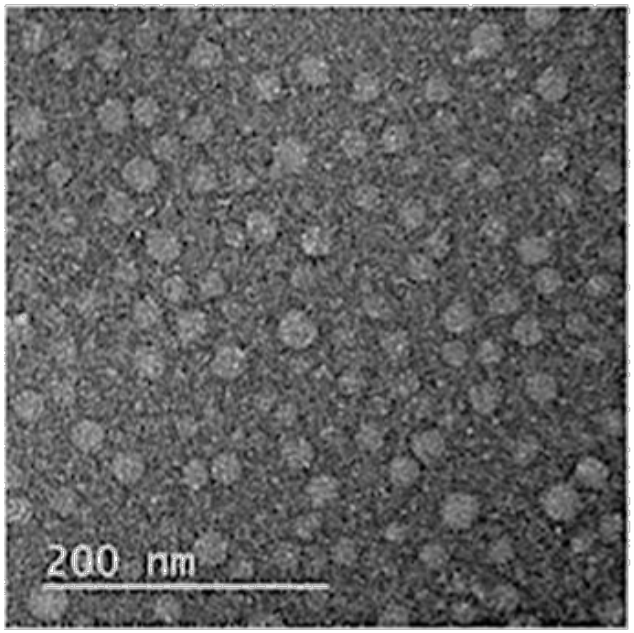


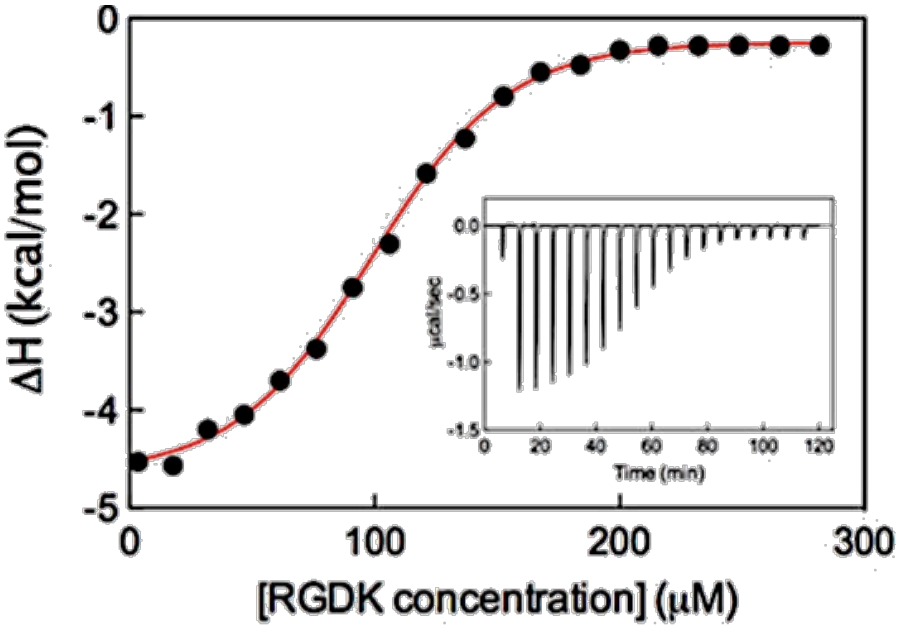






siRNA/**AD**/RGDK formation



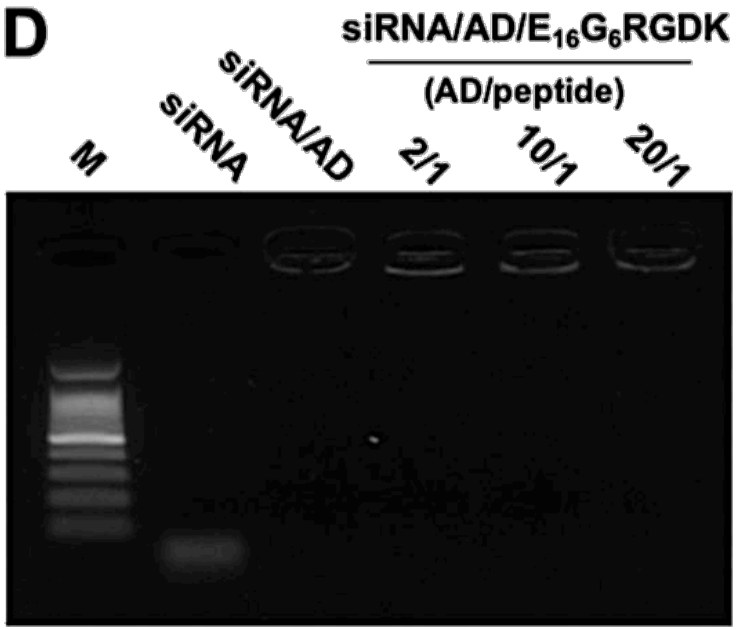


NMs diameter 30-40 nm

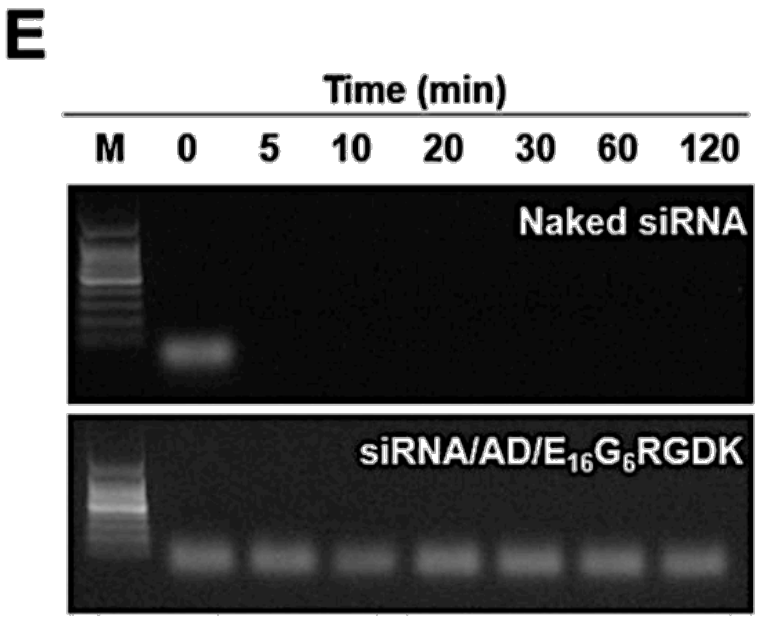
ΔG = -6.8 kcal/mol ΔH = -5.0 ± 0.2 kcal/mol −TΔS = -1.9 kcal/mol

Zeta potential + 15 mV (compared to +25 mV for siRNA/**AD**)

siRNA/**AD**/RGDK: stability and protection



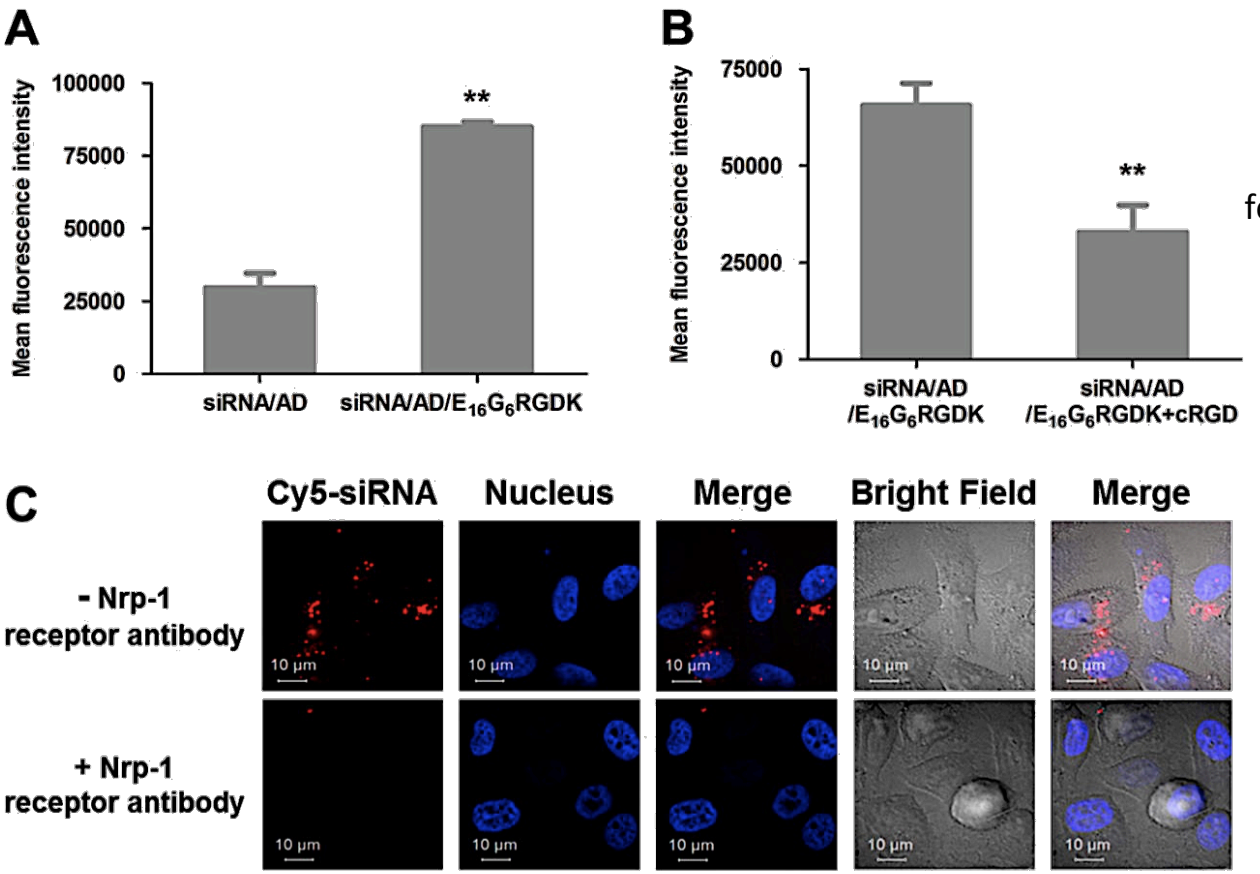
Binding of RGDK does not alter siRNA



Protection from degradation

siRNA/**AD**/RGDK uptake is mediated by integrin and neuropilin-1

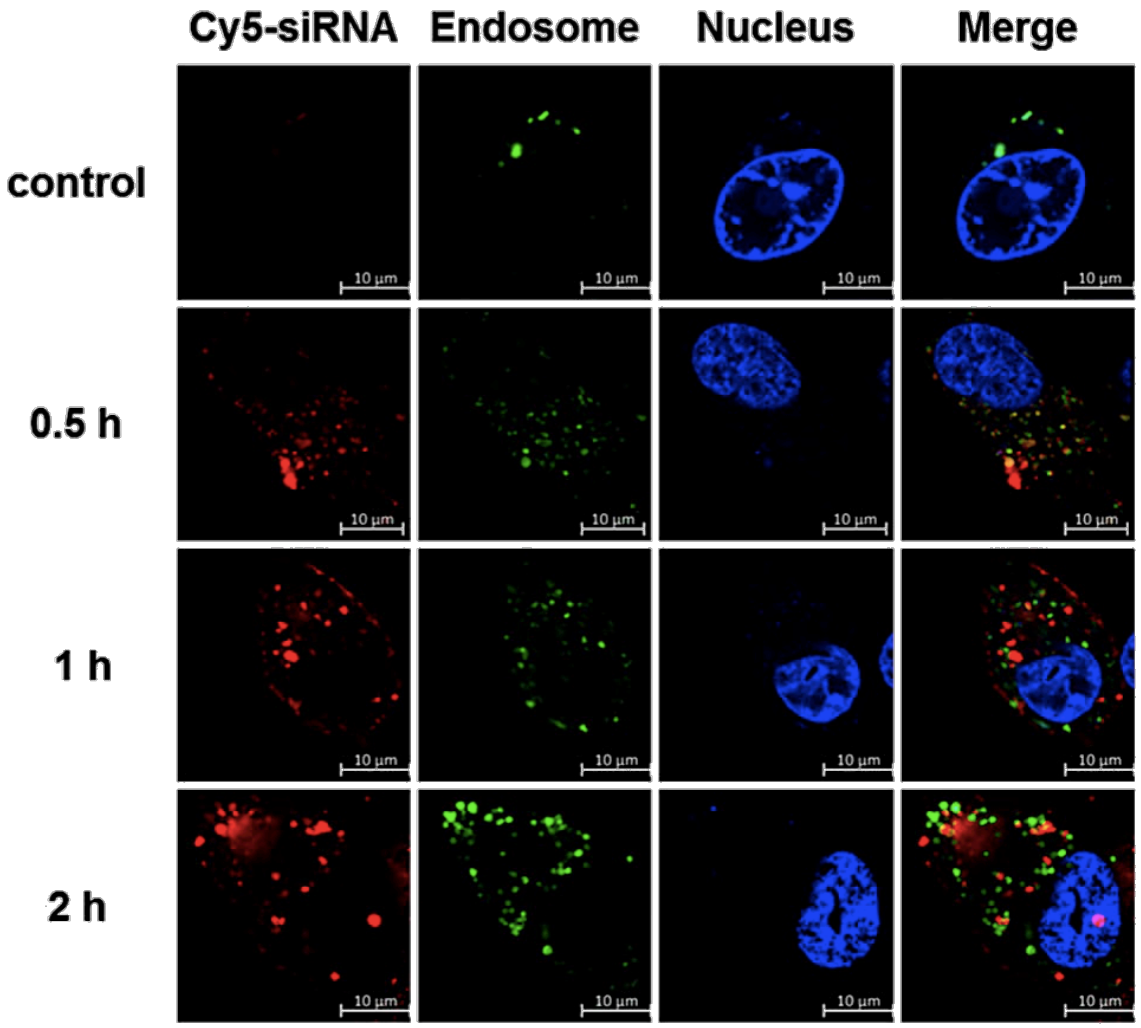
Superior uptake with respect to **AD**



cRGD competes for integrin binding

Blocking Nrp-1 with Mab drastically decreases siRNA/**AD**/RGDK uptake

siRNA/**AD**/RGDK quickly and effectively escapes endosomes

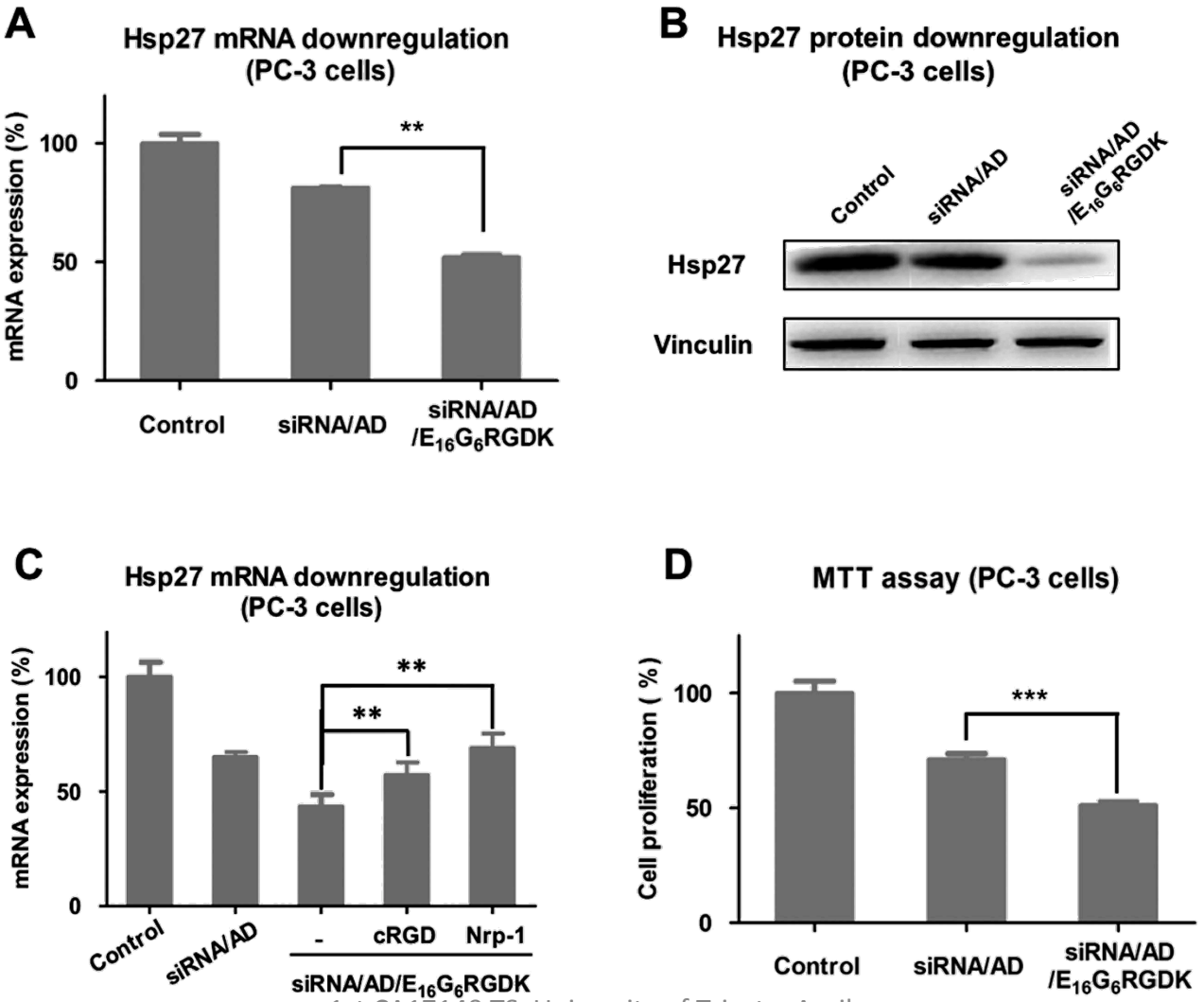


Lyso-tracker

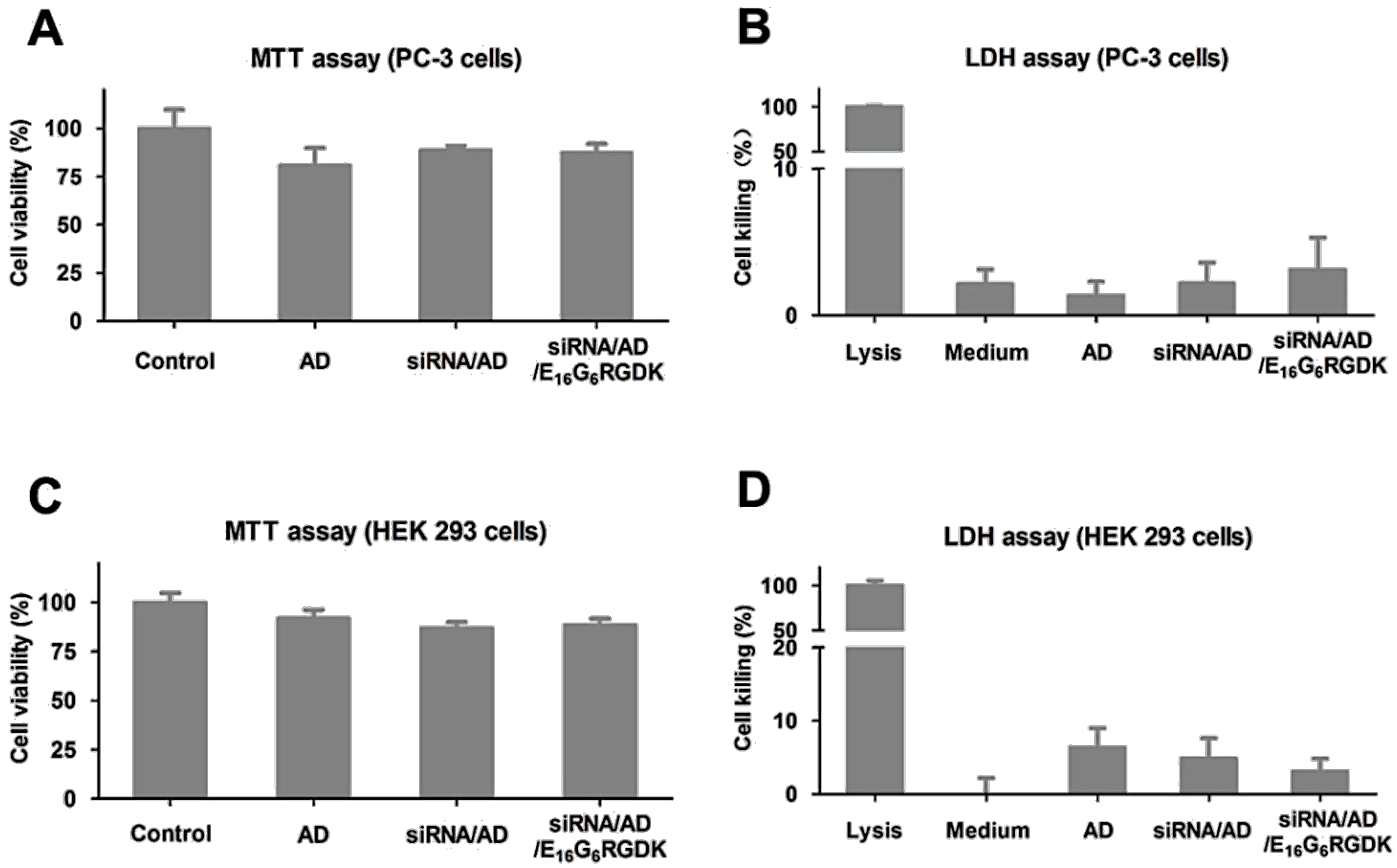
Endosomal escape already in play

Endosomal escape almost finished

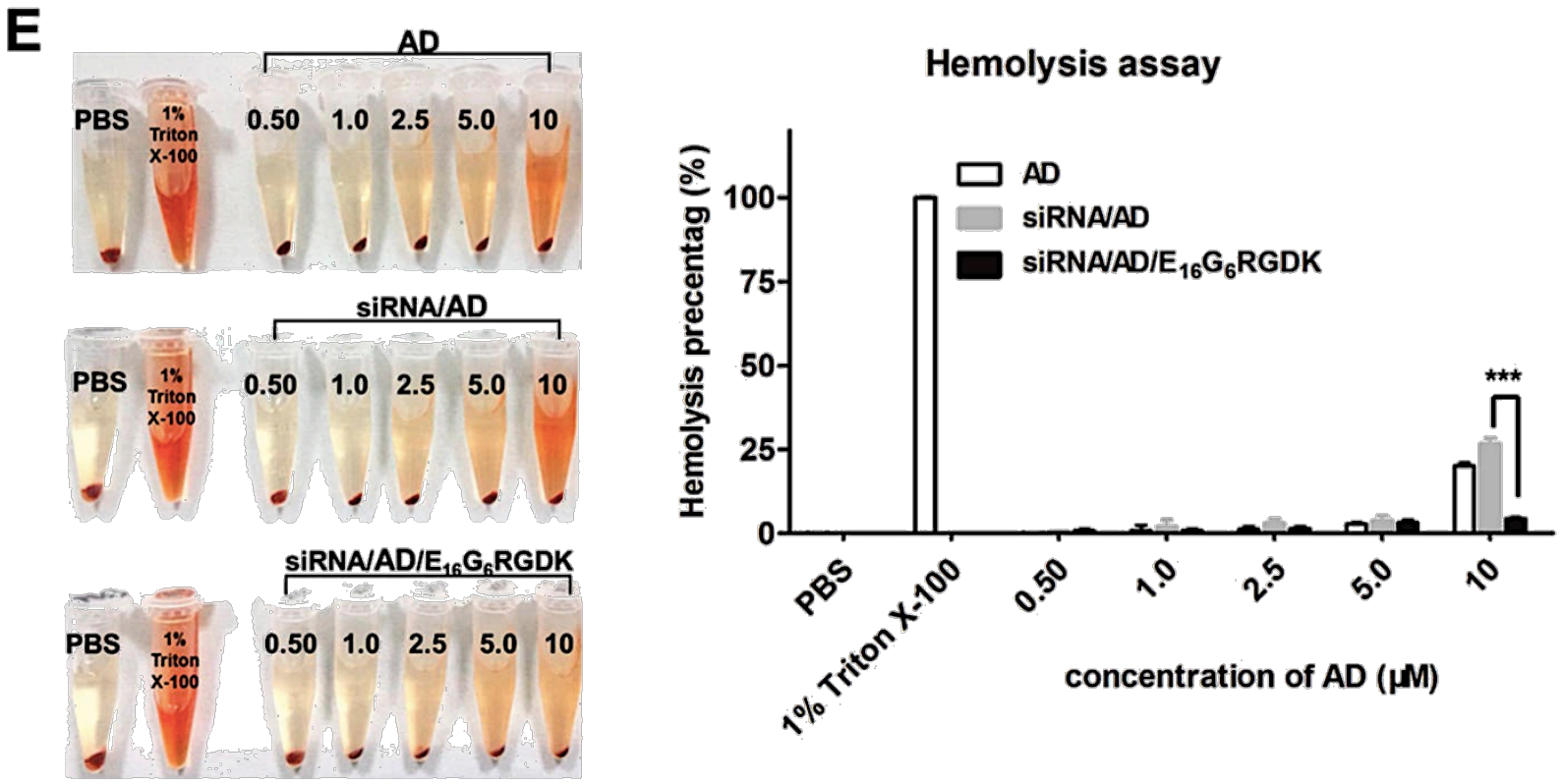
siRNA/**AD**/RGDK is superior to **AD** in gene silencing *in vitro*



siRNA/**AD**/RGDK is not cytotoxic *in cancer and healthy cells*

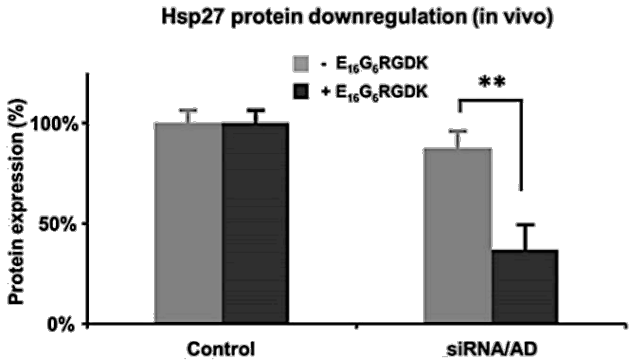


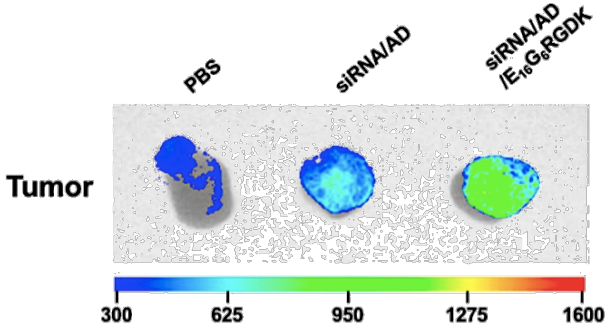
siRNA/**AD**/RGDK has no hemolytic activity

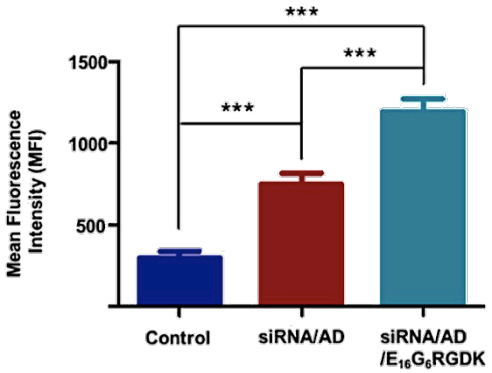


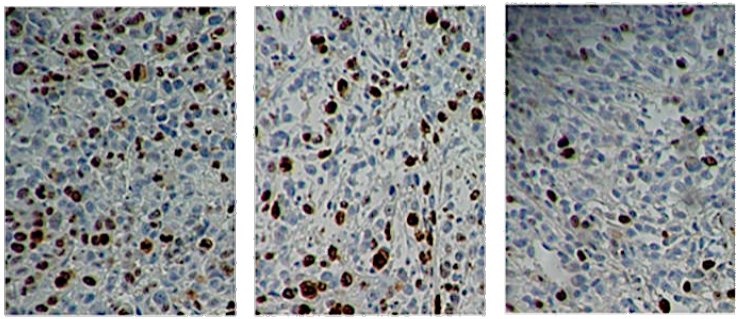
siRNA/**AD**/RGDK is substantially less haemolytic than siRNA/**AD**

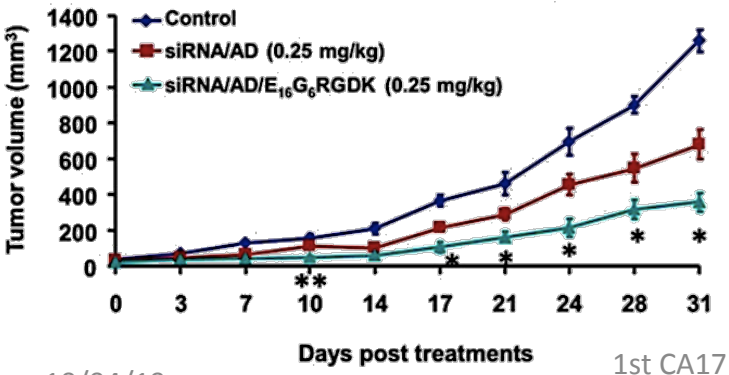
siRNA/**AD**/RGDK is highly effective *in vivo*

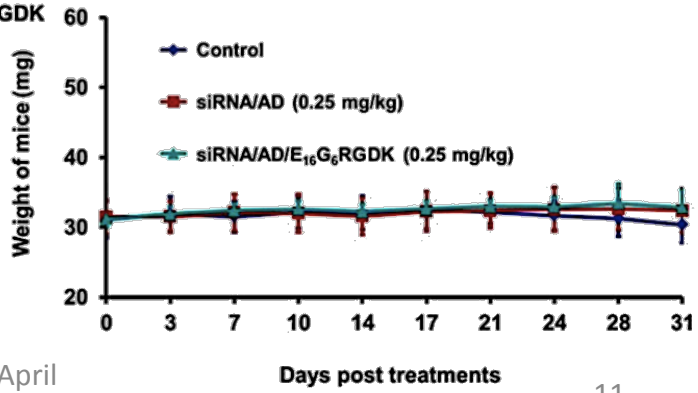












siRNA/**AD**/RGDK is not cytotoxic *in vivo*