# A Novel Platform for Enhanced Intracellular Delivery of Therapeutic Peptides to Promote Tissue Vasorelaxation

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**Statement of Purpose:** Elucidation of the molecular interactions underlying vasoconstriction and its associated pathologies (*e.g.* vascular graft vasospasm and sub-arachnoid hemorrhage) has led to the identification of plethora of promising peptide based therapeutics. However, major barriers exist against therapeutic utilization of intracellular-acting peptides: proteolysis of the peptide in the *in vivo* environment prior to or following cellular internalization, translocation across the the cell membrane, escape from the intracellular endo-lysosomal and exocytosis trafficking pathways, and achieving an effective peptide dose within the intracellular compartment (*i.e.*, cytoplasm) where the therapeutic target is located[1]. To address these delivery barriers, we have synthesized and characterized a library of pH-responsive, endosomolytic polyplex nanoparticles for the intracellular delivery of two peptides that modulate smooth muscle cell physiology by interfering with the p38 mitogen activated protein kinase signaling pathway[2].

**Methods:** A poly(propylacrylic acid) (**PPAA**) homopolymer ($M_n$ = 22,000, PDI = 1.47) was synthesized via RAFT polymerization. A cell penetrant MAPKAP Kinase II inhibitory peptide (**MK2i**)) and a heat shock protein-20 (**HSP20**) peptide mimetic were synthesized through standard FMOC chemistry and purified via reverse-phase HPLC. The MK2i and HSP20 peptides were mixed with the PPAA polymer at a range of charge ratios (CR, defined $[NH_3^+]/[COO^-]$) from 10:1 to 1:10 to form polyplexes. The size and zeta potential of the polyplexes were determined by dynamic light scattering (DLS) analysis. For MK2i nano-polyplexes (**MK2i-NPs**), a CR of 1:3 was chosen as the optimal formulation for further study (hydrodynamic diameter = 110.9 ± 6.89 nm, $\zeta$ = -11.9 ± 3.18 mV). For HSP20 nano-polyplexes (**HSP20-NPs**) a CR of 3:1 was chosen as the optimal formulation for further study (hydrodynamic diameter = 240.9 ± 15.51 nm, $\zeta$ = -0.91 ± 2.56 mV). The pH-responsive, endosomolytic behavior of the polyplexes was assessed through a red blood cell hemolysis assay. The ability of MK2i-NPs and HSP20-NPs to inhibit actin polymerization was assessed with an F-actin stress fiber assay in human coronary artery vascular smooth muscle cells (**HCAVSMCs**) stimulated with angiotensin II (**ANG II**). Human saphenous vein (**HSV**) explants were obtained from consenting patients for the evaluation of MK2i-NP mediated vasorelaxation: 1 mm HSV rings were treated for 2 hours with MK2i-NPs, MK2i peptide, or controls and subsequently contracted with phenylephrine (**PE**, $10^{-6}$ - $10^{-7}$ M) and relaxed with cumulative log doses of sodium nitroprusside (**SNP**) to determine % relaxation. Rat aorta smooth muscle was harvested from fresh cadaver hearts from discarded animals from the Vanderbilt Medical Center: 1 mm aortic rings were treated for 30 mins with HSP20-NPs, HSP20 peptide, or controls and subsequently contracted with phenylephrine ($5*10^{-8}$ M) to determine % inhibition of contraction vs. pretreatment.

**Results:** NPs showed no hemolytic behavior at pH 7.4 or 6.8 but showed switch-like, robust hemolysis at pH 6.2 and 5.6, suggesting that NPs will enable peptide cytosolic delivery. Actin stress fiber assays with both MK2i-NPs and HSP20-NPs showed enhanced inhibition of stress fiber formation compared to the peptide alone (**fig. 1A)**. HSV explants treated with MK2i-NPs showed significantly more relaxation than the peptide alone, and achieved the same level of relaxation at 1/10$^{th}$ of the dose of peptide (100 μM vs 10 μM, **fig. 1B**). HSP20-NPs displayed a 5-fold increase in inhibition of contraction of rat aortic smooth muscle compared to the HSP20 peptide alone (500 μM vs 100 μM, **fig. 1C**).

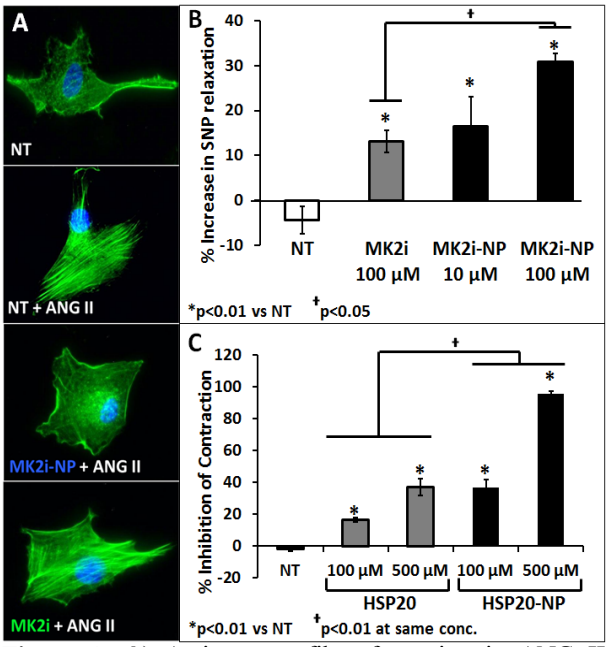


**Figure 1: A**) Actin stress fiber formation in ANG II stimulated HCAVSMCs treated with MK2i or MK2i-NPs **B**) HSV SNP induced relaxation following 2 hr treatment with MK2i or MK2i-NPs. **C**) Inhibition of PE induced contraction in rat aortic smooth muscle following 30 min treatment with HSP20 and HSP20-NPs.

**Conclusions:** We have developed a platform for enhanced intracellular delivery and bioactivity of therapeutic peptides to treat vasoconstriction with pH-responsive, endosomolytic nano-polyplexes. We have proven the ability of these polyplexes to modulate smooth muscle physiology *in vitro* and *ex vivo*. Our results suggest that these nano-polyplexes present a promising approach to the intracellular delivery of peptide-based therapeutics to treat vasospasm in vascular pathologies.

**References:**

**[1]** Li et al. *Curr Pharm Design.* 2011;17(3):293-319.
**[2]** Cornelissen et al. *ATVB.* 2004;24:451-456.