Does the architecture of cell-penetrating peptides influence cell interactions?

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Efficacious delivery of emerging therapeutic compounds to treat diseases such as cancer remains limited by our understanding of interactions between drug carriers at the nano-bio interface. Cationic cell penetrating peptides (CPPs) are reported to enhance the cellular absorption of therapeutic compounds. Our innovative strategy is to decorate nanoparticles with CPPs, facilitating enhanced delivery of unaltered, active drug. Key interactions between CPPs and the cell membrane that influence uptake are not yet fully understood. Predominant theories have identified peptide concentration, charge and amino acid sequence[1] as key drivers. We anticipate CPP architecture to play a crucial role, but it has not been explored systematically in this context.

We have produced novel, highly-defined poly(lactic-*co*-glycolic) acid (PLGA) nanoparticles functionalized with CPPs using microfluidics. CPPs of different architectures (short, long linear and branched), including the well-studied TAT, were designed for conjugation to PLGA nanoparticles (Figure 1A). Investigations of cell uptake using flow cytometry revealed that internalization of all CPP nanoformulations was low, with TAT-tagged nanoparticles being greater than nanoparticles tagged with CPPs of short and branched architecture. This data supports the hypothesis that the amino acid sequence of CPPs is an important driver of cell interactions. To better understand the low overall uptake, the initial interactions of the CPP-tagged nanoparticles with cells were of interest. Using single particle tracking we have followed the behaviour of CPP-tagged nanoparticles after 2 min incubation at the nano-bio interface. We observed localisation of the nanoparticles in close proximity to the cell membrane (Figure 1B). These results provide insights into the interactions of CPP-tagged nanoparticle formulations that govern cell uptake of nanomedicines.

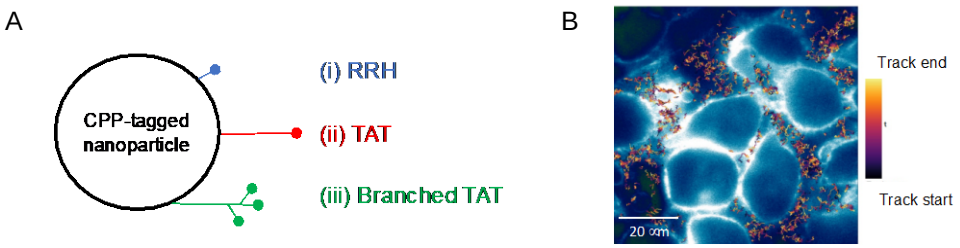

Figure 1: (A) Schematic diagram of CPP-tagged PLGA nanoparticles. CPPs of different architecture (i) short arginine-arginine-histidine (RRH) (ii) long linear trans-activating transcriptional activator (TAT) (iii) TAT with three terminal RRH groups (B) Single particle tracking of fluorescently-labelled PLGA nanoparticles during incubation with HeLa cells. Different colours indicate tracking time.

References

1. Foged C, Nielsen HM. Cell-penetrating peptdies for drug delivery across membrance barriers. *Expert Opinion on Drug Delivery* 2008;5:105-17.