

**Combinatorial design of nanoparticles for efficient delivery of therapeutic biomacromolecules through the blood brain barrier**

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The blood-brain barrier (BBB) is a crucial biological filter made of endothelial cells mediating the communication between the blood and the central nervous system. This unique barrier effectively blocks the entry of harmful substances from the bloodstream. However, it also imposes strict limitations on the passage of therapeutic proteins, e.g., enzymes and monoclonal antibodies, thereby impacting their therapeutic benefits when targeting the brain. To address this challenge, a variety of nanoparticulate drug transporters have been designed and several targeting moieties have been shown to favor their transport through the BBB. However, there is a lack of a systematic study that enables the screening of optimized parameters for nanoparticles (NPs) in designing such carriers. Herein, we developed a novel method of NP combinatorial surface modification by utilizing copper(I)-catalyzed azide alkyne cycloaddition reaction. This method enables an efficient chemical modification of the surface of NPs with a variety of ligands for facilitating screening experiments. In specific, these NPs consist of a silica core with an immobilized model protein, protected in an organosilica shield. For investigating the capability of the combinatorially modified NPs to cross the BBB, we utilized an *in vitro* BBB model system. The results showed that the NPs exhibited good biocompatibility when incubated with human brain endothelial cells (hCMEC/D3) for 24 hours. In addition, screening experiments based on this novel method identified lead formulations with combinatorial modification of glucose, transferrin and carboxy-, amino-functions, which significantly increased delivery efficiency without compromising BBB integrity. Taken together, we have established a platform that enables to investigate the impact of NPs' surface modification on their interactions with cells. This approach provides a valuable avenue for screening and selecting formulations with improved delivery efficiency.