Semi-Randomized Zwitterionic Peptides to Prevent Nanoparticle Fouling

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Introduction: Nanoparticles (NP) are a clinically proven siRNA delivery platform [1]. However, recent data indicate that NPs exhibit poor systemic delivery due to protein adsorption-mediated opsonization and accelerated clearance via macrophages [2]. Anti-fouling NP modifications such as poly(ethylene glycol) (PEG) and zwitterionic (ZI) polymers and peptides have been shown to be highly efficacious but are susceptible to immunological reactions due to consumer product exposure and repeated chemical structures [2]. As an alternative, we propose the use of computationally designed semi-randomized ZI peptides (srZIPs) synthesized via controlled random amino acid (AA) incorporation in a single sequence to produce libraries (10^3-10^6) of related peptides to modify NP. We hypothesize that srZIPs with low predicted protein interaction will provide anti-fouling characteristics to NP and also be immunologically unique, hindering adaptive immune reactions. To investigate these hypotheses, the design, synthesis, and characterization of srZIP-NPs in vitro and in vivo were explored.

Materials and Methods: srZIPs of various AA lengths were generated in silico and scored using an algorithm based upon a peptide-peptide interaction model (PASTA) [3] for lowest interaction potential ($\Delta G > 2$ kJ/mol) for anti-fouling (AF) srZIPs, and high interaction potential ($\Delta G < -3$ kJ/mol) for pro-fouling control srZIPs. srZIPs were synthesized via solid phase peptide synthesis, with mixtures of AA precursors for semi-randomization, and confirmed through mass spectrometry. NPs were assembled from diblock copolymers of a poly(dimethylaminoethyl methacrylate) (DMAEMA) first block and 25% DMAEMA, 50% butyl methacrylate, and 25% propylacrylic acid second block synthesized via reversible addition fragmentation chain transfer (RAFT) polymerization (**Fig 1A**) [4]. srZIP or 5 kDa PEG were conjugated to NP via carbodiimide chemistry (**Fig 1B**) and confirmed via fluoraldehyde assay and NMR. NP aggregation in serum and plasma was evaluated via dynamic light scattering (DLS); adsorbed protein was quantified using the bicinchoninic acid assay. NP-mediated siRNA knockdown of GAPDH in mouse mesenchymal stem cells (MSCs) was assessed via RT-PCR. Macrophage and MSC uptake of NP was evaluated by flow cytometry and in vivo pharmacokinetics were characterized via intravital fluorescent microscopy of Cy7 labeled NPs delivered via retro-orbital injection in BALB/c mice. Fluorescence intensity profiles were fitted to 2 compartment pharmacokinetic models using GraphPad Prism.

Results and Discussion: srZIP-NPs maintain similar size of ~ 25 nm to unconjugated NPs in PBS, while PEG-NP diameter increases to 40 nm. srZIP-NP and PEG-NPs have a dramatic >14-fold reduced aggregate size compared with unmodified NPs. Protein adsorption by anti-fouling srZIP-NP is reduced by 65% compared to NP and 37% to PEG-NP, while pro-fouling srZIP-NPs adsorb 70% more protein than unfunctionalized NP (**Fig 1C**). Anti-fouling srZIP-NP uptake by macrophages is reduced by 25% compared to NPs while PEG-NP uptake is reduced 75%. Anti-fouling srZIP-NP circulation time was increased 5-fold over NPs, with a half-life of 32 minutes (**Fig 1D**).

Conclusions: Data suggest that low interaction potential srZIP functionalization provides anti-fouling properties to NPs, reducing protein adsorption and macrophage uptake and enhancing systemic circulation time. In contrast, high interaction potential srZIPs conjugated to NPs potentiate protein interaction. Future work will focus on evaluating in vivo biodistribution and immunological behavior of srZIP-NPs after repeated intravenous administration. Funding: F31AR076874, T32GM118283, R01DE018023, R01AR056696, DMR-2103553

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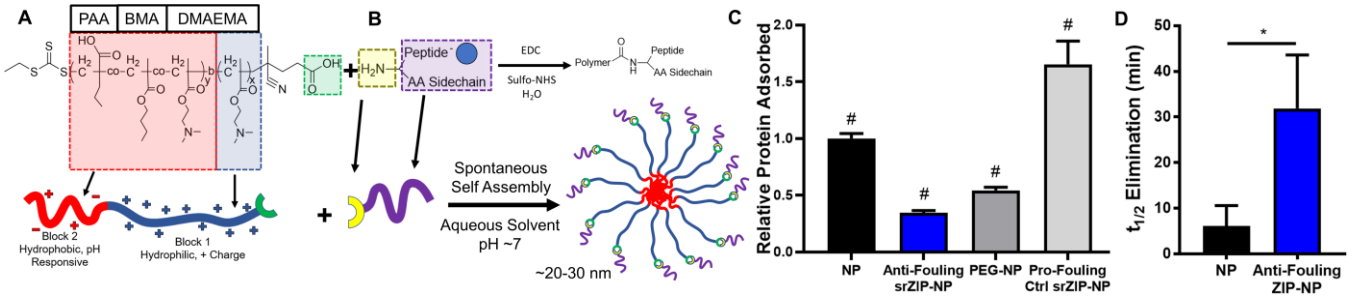

Figure 1: A: Overview of NP synthesis. **B**: Overview of peptide conjugation. **C**: Relative protein adsorption in 10% FBS. # indicates $p < 0.05$ vs all other conditions using 1-way ANOVA with Tukey's post-hoc. **D**: Circulatory elimination half-life in mice after retro-orbital injection. * indicates $p < 0.05$ using t-test.