Directed discovery of high-loading nanoaggregates enabled by drug-matched oligo-peptide excipients

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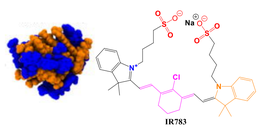
Introduction

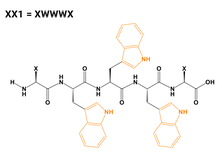
The assembly of drugs as structural components into supramolecular carriers is an attractive alternative to sequential incubation of drug into pre-designed carriers that can significantly increase loading. However, little is known about the process of drug aggregate formation or how to predict co-assembly of particular drugs and excipients. Peptides offer the opportunity to probe for motifs that may be important in self-assembly due to their wide chemical space, facile synthesis, and biodegradable nature[1]. To this end, a series of tryptophan based pentapeptides with potential drug-co-assembly capability were synthesized, systematically differing in charge, aromatic spacing, and rigidity. The peptides were screened for co-assembly with a library of 20+ drugs covering a wide chemical space. The library candidates were characterized by DLS, enhancement of optical density of the drug in aqueous solutions. Several peptide/drug combinations were identified that formed readily dispersible nanoparticles with over 95% drug loading and favorable release kinetics.

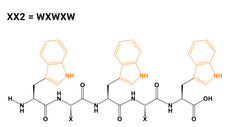
Rational Design of Peptide Nanocarrier Excipients

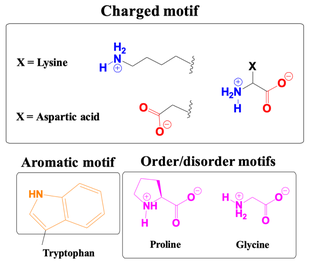
Peptides were designed based on mimicry of IR783, a dye that has been shown to co-assemble to form nanoparticles with various drugs leading to very high loading and effective delivery[2]. Inspired by this previous work, two pentapeptide scaffolds with differing arrangement of tryptophan were selected for the study. Furthermore, aspartic acid, lysine, proline, and glycine were used to assess the effect of order and charge.

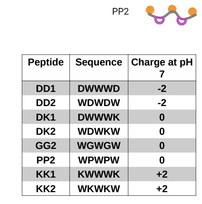


Screenshot of MD simulation of IR783 and the drug Sorafenib, taken from Shamay, etal[1]. Blue is dye, orange is drug.

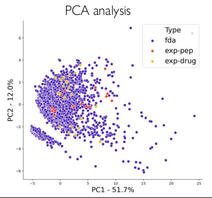


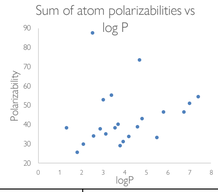


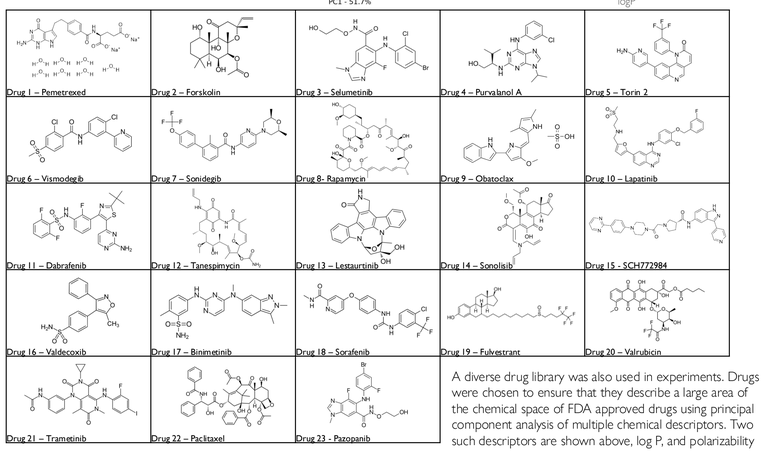




Peptide	Sequence	Charge at pH 7
DD1	DWWWD	-2
DD2	WDWDW	-2
DK1	DWWWK	0
DK2	WDWKW	0
GG2	WGWGW	0
PP2	WPWPW	0
KK1	KWWWK	+2
KK2	WKWKW	+2

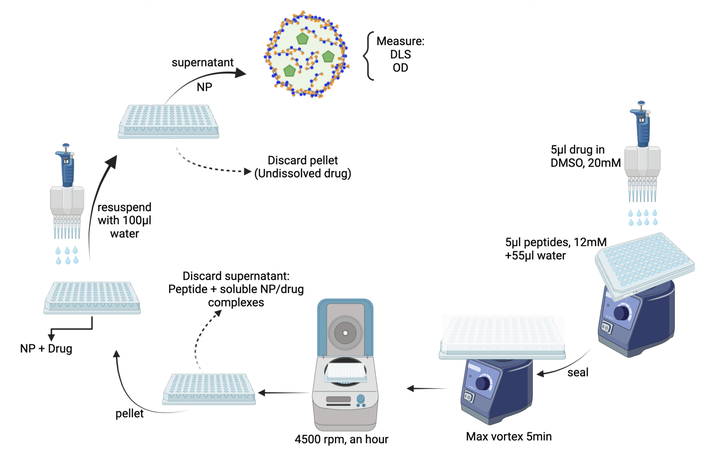




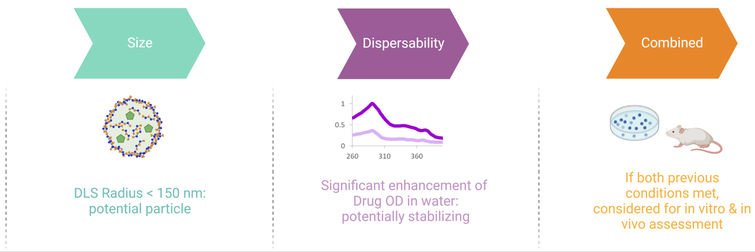


A diverse drug library was also used in experiments. Drugs were chosen to ensure that they describe a large area of the chemical space of FDA approved drugs using principal component analysis of multiple chemical descriptors. Two such descriptors are shown above, log P, and polarizability

Drug-Peptide Assembly Screening Assay

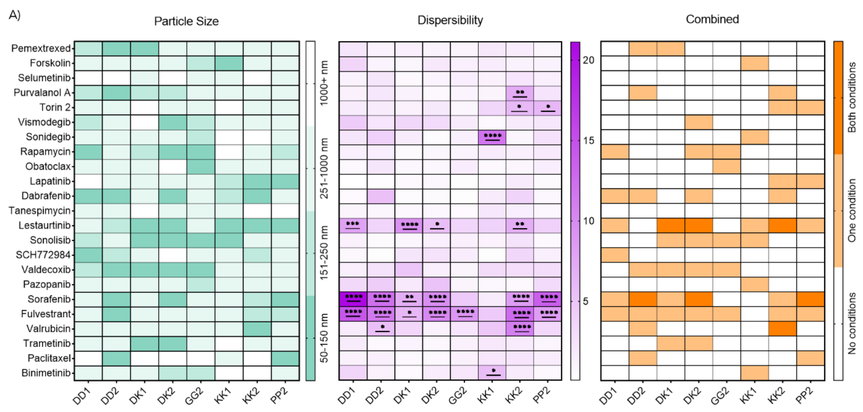


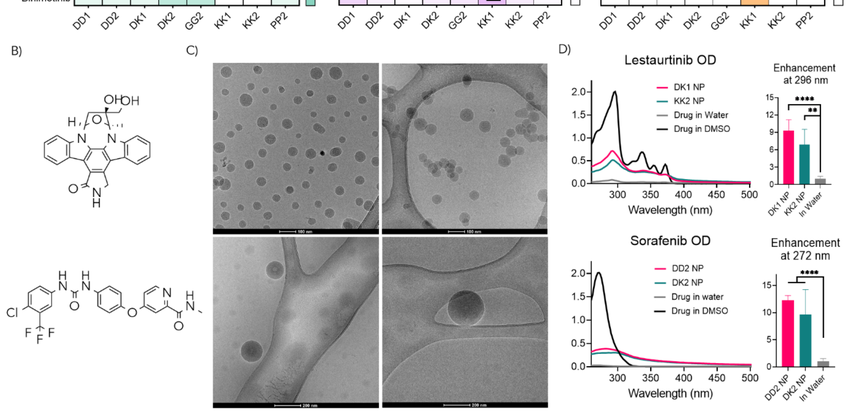
High-Throughput Screening Metrics



Screen Results in Multiple Drug-Peptide Nanoparticle Hits

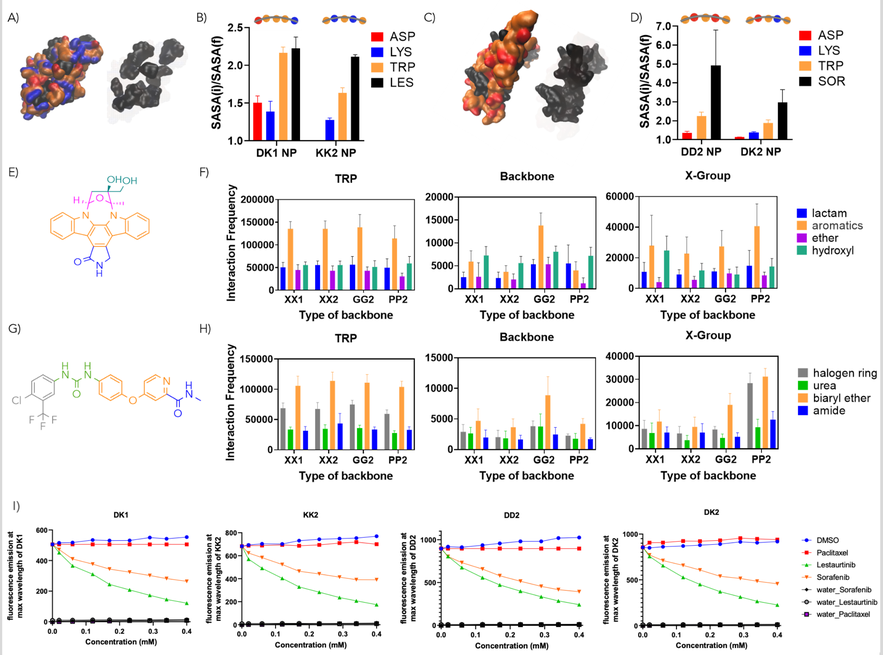
Two metric assay able to identify formulations that can be scaled up with small sizes and good drug encapsulation



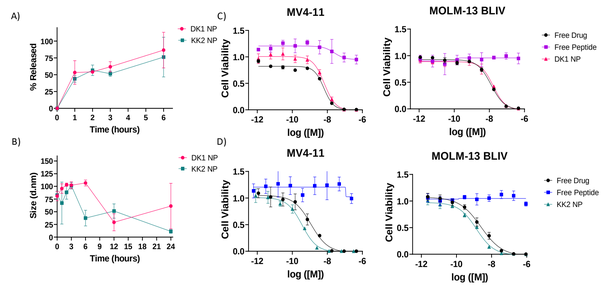


Statistical significance was calculated for enhancement with 2-way Anova method using Prism software.

Molecular Dynamics Simulations and Experimental Validation

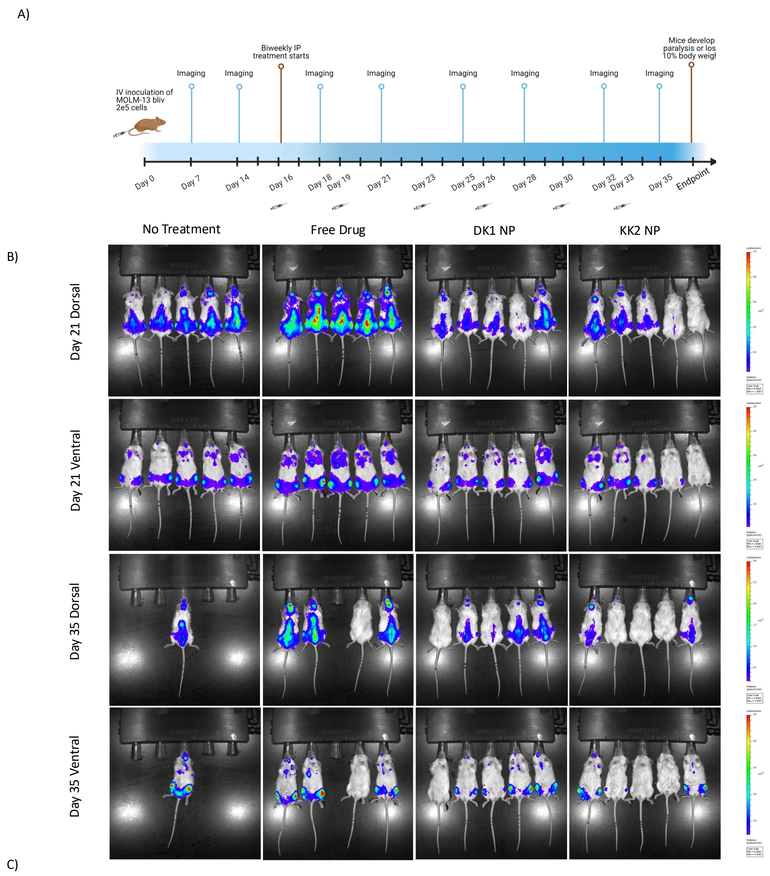


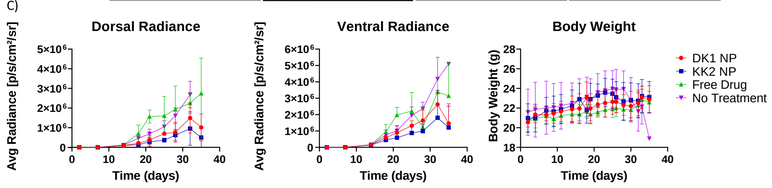
In Vitro Cancer Cell Inhibition



A-B) The particles remain well dispersed in serum and release cargo within 6 hours
C-D) Free peptide is not toxic. Particles are as effective (DK1 NP) or more effective (KK2 NP) than free drug

Nanoparticle Improves Leukemia Treatment over Free Drug





Both NP groups significantly inhibit tumor burden and are none toxic to mice

Discussion

We were able to develop an experimental assay that could identify high potential peptide drug combinations for the formation of drug nanoparticles. These particles are primarily composed of drug (+90%). The two-metric approach identified 7 hits out of 184 combinations. Two of these were selected for scale up and demonstrated agreement with the high-throughput results. Molecular dynamics simulations and ToF SIMS confirmed a core-shell structure of nanoparticles. Overall, it appears that the XX2 platform had more hits with the current library. In vitro and in-vivo data support increased efficacy of treatment in nano-formulations in acute myeloid leukemia models3.

Future Work

The limitations of this study include a generally small library size. We can start modeling interactions with chemical interactions to predict outcomes with Machine Learning, where the current data serves as a training set. A larger library would be required to validate the data to ensure that the modeling is robust.

References

1. Bobo, D., Robinson, K. J., Islam, J., Thurecht, K. J. & Corrie, S. R. Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date. Pharmaceutical Research 33, 2373–2387 (2016).
2. Shamay, Y. et al. Quantitative self-assembly prediction yields targeted nanomedicines. Nature Materials(2018) doi:10.1038/s41563-017-0007-z.
3. Berisha, N., Farahpour, A. et al. Chem. Accepted.

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