Optimizing supramolecular and chemical stability to enhance the design of self-assembling prodrugs

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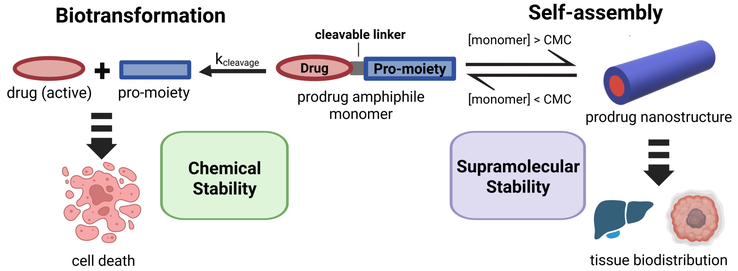
Introduction

- Efficacy of **traditional prodrugs** including **antibody-drug conjugates (ADCs)** relies on **linker chemical stability**
- Disassembly of **self-assembling prodrugs (SAPDs)** into the prodrug monomer depends on **nanostructure supramolecular stability**
- Thus, both supramolecular and chemical stability play crucial roles in determining the antitumor efficacy and toxicity of SAPDs
- Precise relationship between these two factors remains largely unexplored



Scheme 1: Illustrative relationship between chemical and supramolecular stability and the pharmacokinetics/pharmacodynamics of SAPDs

Methods

Design of CPT SAPDs with different chemical stability

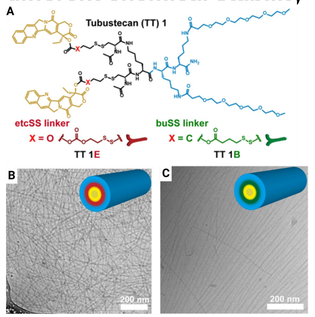


Figure 1: A) Chemical design of tubustecan (TT)[1] monomers with **different disulfide linker** and **B, C**) representative cryo-EM images

Design of CPT SAPDs with greater supramolecular stability

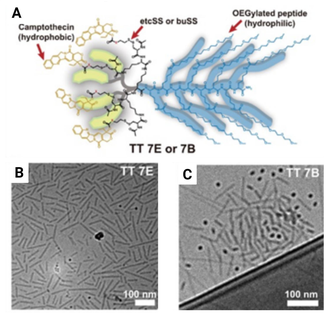


Figure 2: A) Chemical design of TT monomers with **higher OEGylation and CPT content** and **B, C**) representative TEM images

Evaluation of *in vitro* and *in vivo* efficacy

- ***In vitro***: drug release with and without GSH, IC_{50} measurement
- **Antitumor efficacy**: inhibition in HT-29-bearing nude mice, i.v. injection
- **Systemic toxicity:** maximum tolerated dose (MTD) measurement

Chemical Stability Dominates Pharmacodynamics

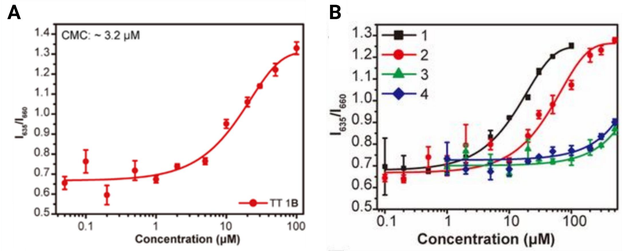


Figure 3: Critical micellization concentration (CMC) study using the Nile Red assay for **A**) **TT 1B** and **B**) **TT 1E (1)**, as part of a previously conducted study by Hao Su et al., ***PNAS***, 2020 [Ref 2]

- **TT 1B** and **TT 1E** have comparable CMC (3.2 μM vs 2.7 μM[2])
- The two SAPDs have **similar supramolecular stability**

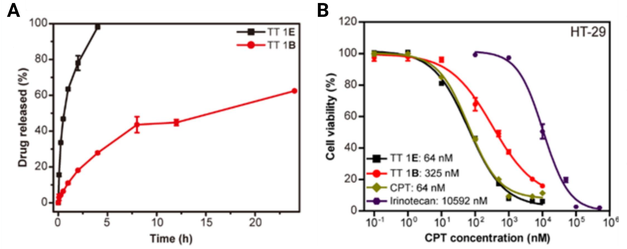


Figure 4: A) Cumulative release plot of free CPT from **TT 1E** and **TT 1B** solutions (200 μM) in 1x PBS containing 10 mM GSH at 37°C and **B**) representative IC_{50} measurement study with HT-29 colon cancer cells

- **TT 1E** has much faster drug release than **TT 1B**
- **TT 1E has lower chemical stability**
- **TT 1E** has much lower IC_{50} than **TT 1B** (64 nM vs 325 nM)
- **Lower chemical stability increases cytotoxicity**

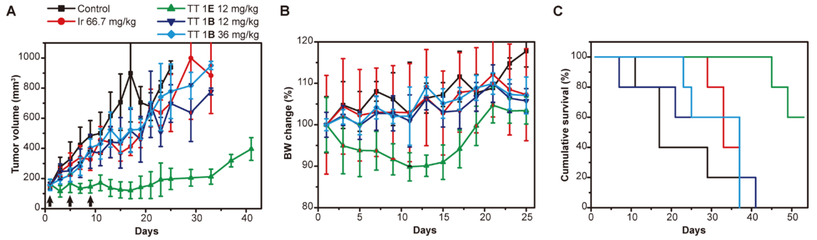


Figure 5: Comparison of *in vivo* antitumor efficacy of **TT 1E** and **TT 1B** in nude mice bearing HT-29 tumors. **A**) Tumor volume, **B**) body weight, and **C**) cumulative survival were monitored and plotted

- Only **TT 1E** exhibited significant tumor growth inhibition and prolonged survival
- **Lower chemical stability translates to increased antitumor efficacy**

Supramolecular Stability Dominates Pharmacokinetics

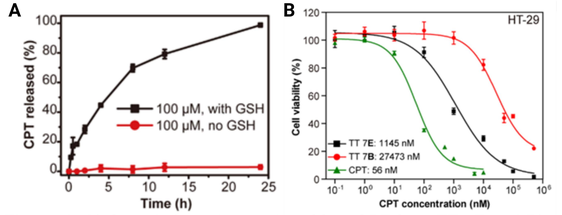


Figure 6: A) Cumulative release plot of free CPT from **TT 7E** and **B**) representative IC_{50} measurement study with HT-29 colon cancer cells

- **TT 7E** has slower drug release than **TT 1E**, but faster than **TT 1B**
- **TT 7E** likely has higher supramolecular stability than **TT 1E**
- **Chemical stability has dominant influence on drug release kinetics and cytotoxicity**

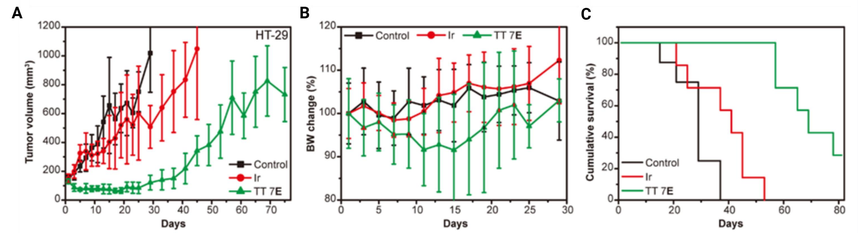


Figure 7: Comparison of *in vivo* antitumor efficacy of **TT 7E** and **irinotecan (Ir)** in nude mice bearing HT-29 tumors. **A**) Tumor volume, **B**) body weight, and **C**) cumulative survival were monitored and plotted

- **TT 7E** has lower MTD than **TT 1E** (15 mg/kg vs 24 mg/kg[2])
- **TT 7E** exhibited longer tumor growth inhibition and prolonged survival than **TT 1E**
- **Higher supramolecular stability translates to increased antitumor efficacy and toxicity**

Conclusion

- **Both TT 1E and TT 7E outperformed irinotecan**, a clinically approved CPT prodrug
- **Higher supramolecular stability** increases nanostructure integrity in circulation and higher accumulation in both the target site and healthy tissue leads to **greater therapeutic efficacy but also systemic toxicity**
- **Lower chemical stability** facilitates more effective drug release at the target site and **enhanced therapeutic efficacy**

Significance: This work provides critical insight into the design principles of supramolecular drug delivery systems and their potential clinical translation.

References

(1) Hao Su et al., *J Am Chem Soc.,* 2019:17107-17111
(2) (2) Hao Su, Feihu Wang et al., *P Natl Acad Sci USA.*, 2020:4518-4526.

Acknowledgement

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