FOUNDATIONAL SYNTHESIS OF NLS PEPTIDES FOR Tb-155 RADIOLABELING

Looking towards Radiopharmaceutical Production

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Motivation

The Auger Electron Promise

- Auger electrons have very short range (nm- μm) and low energy (< 1 keV) [1]
- Cascade effect: High yield
- Highly localized energy deposition (~10 Gy)
- Normal tissue sparing [8]
- High tumor cytotoxicity

- **Highest damage observed when IN the nucleus**

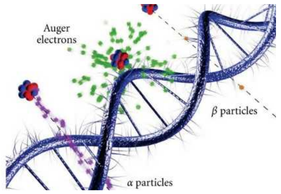
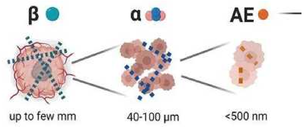


Figure 1: Auger electron therapy compared to alpha and beta therapies. (Hererro et al. 2021)



Bolcean et al. (2023)

- Minimized normal tissue damage and cross-dosing observed with AE emitters than with α and β emitters
- Selective cell delivery
- Potential to revolutionize Targeted Radionuclide Therapy (TRT)

Challenges in proving clinical effectiveness of AE emitters
- Successful delivery of AE emitter to nucleus
- Effective nuclear delivery mechanisms

→ NLS Peptides

Targeting Tumors on the DNA Scale

Long Term Goal: Develop a dual-function molecule for the selective delivery of Tb-155 into the nucleus of a prostate cancer cell

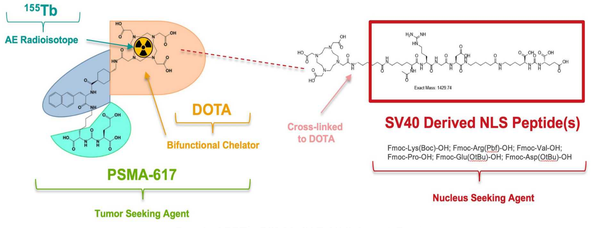


Fig. 1: 155Tb-PSMA-617-NLS Agent Design

Nuclear Localization Sequence (NLS) Peptides
- Mediate the transport of proteins from cytoplasm into nucleus
- Used in TRT to carry drug cargo into nucleus

- Signal to nuclear transporters to help NLS containing molecule through the nuclear pore complex to the nucleus
- NLS recognized by nuclear transporters which interact with nucleoporins to help NLS molecule to nucleus through the nuclear pore complex (cite) (lu)

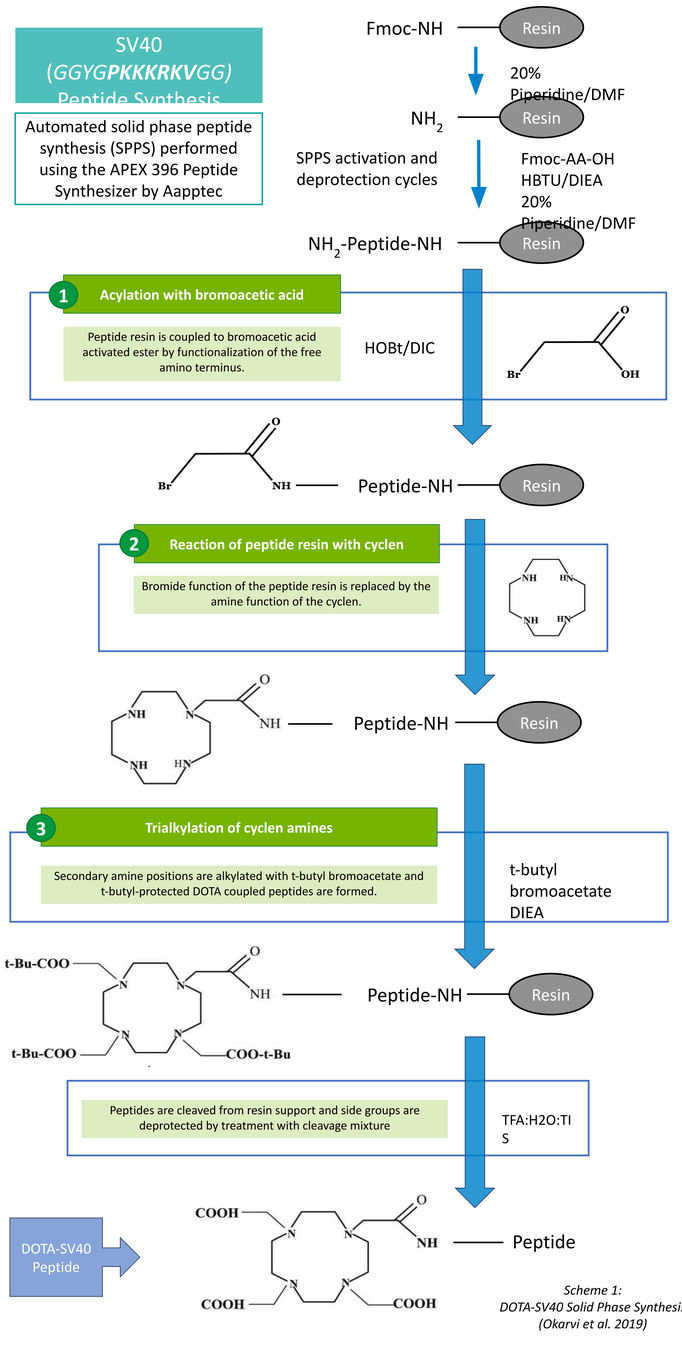
- Early work with ^{111}In-trastuzumab-NLS demonstrated some cytotoxicity in breast cancer cells [3,4]

Design and synthesis of an NLS peptide for effective nuclear import and radiopharmaceutical delivery

Methods

SV40 (*GGYGPKKKRKVGG*) Peptide Synthesis

Automated solid phase peptide synthesis (SPPS) performed using the APEX 396 Peptide Synthesizer by Aapptec

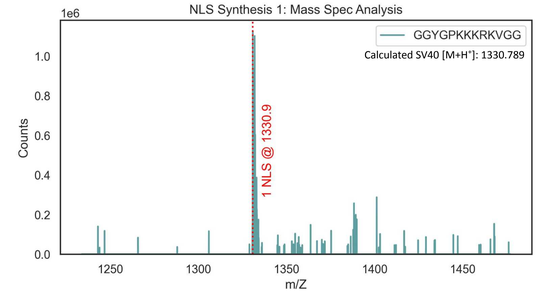


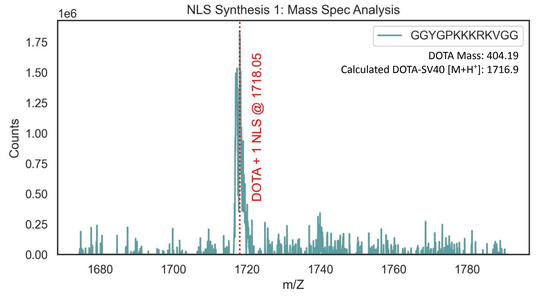
Scheme 1: DOTA-SV40 Solid Phase Synthesis (Okarvi et al. 2019)

Results

Mass Spec

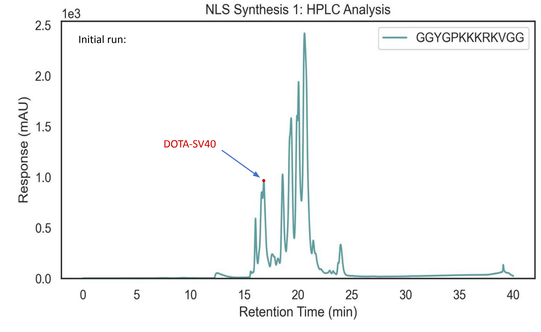
Structural identity analysis

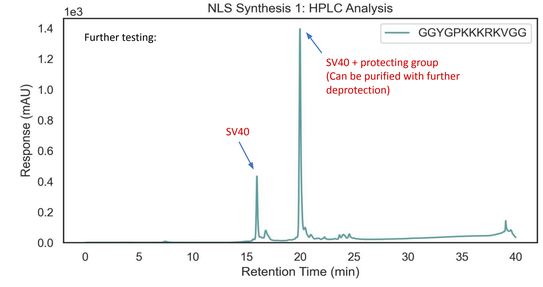




HPLC

Purification analysis and fractionation





Significance

Successful Synthesis

- Confirmed synthesis of SV40 peptides and crosslinked DOTA-SV40 with >90% purity

- Densities at
  - m/Z = 1330.9: one SV40 NLS peptide
  - m/Z = 1718.05: DOTA-SV40 crosslinked peptide with NLS conjugation

A Novel Approach

- Novel, ground up synthesis of SV40 *GGYG**PKKKRKV**GG* peptide and AE delivery mechanism allows for fine tuning of sequence in response to specific targets and controlled, "hands on" adaptive modular synthesis
- Utilizing peptide-based cellular targeting vectors rather than antibody-based cellular targeting vectors which are less sensitive *in-vivo* to hostile environments of high pH or high temperature and thus more resistant to breakdown
  - Improved targeting with higher probability of internalization and rapid system clearance

- **Paving the way for increased efficiency in AE radionuclide drug cargo delivery**

Future Work

Optimization of AE-Radiopharmaceutical

- Synthesis and study of many other NLS sequences for bioconjugation
- Novel NLS sequences may offer increased nuclear import and subcellular accumulation [5]

NAME	SEQUENCE	NET CHARGE	MASS
SV40 NOW	*CGYGPKKKRKVGG*	5	883.13
EGL-13	***MSRRRKANPTKLSENAKKLAKEVEN***	6	2899.35
c-Myc	CGYG**PAAKRVKLDS**	2	997.19
NLP	**AVKRPAATKKAGQAKKKKLD**	9	2137.58
TUS	**KLKIKRPVK**	5	1109.45
SV40 (extended)	**SSDDEATADSQHSTPKKKRKVEDPYC**	0	3148.39
HTVL	MPKTRRRPRRSQRKRPPTPWAHFPGFGQSLC	8	3733.37

Table 1: Proposed NLS Sequences [2,11]

Next Steps

- Tb-155 radiolabeling studies
- Conjugation of DOTA-SV40 to protein-based cell targeting vector PSMA-617
- Confirmation of improved efficiency of AE nuclear delivery and characterization of ^{155}Tb-PSMA-617-NLS therapeutic effectiveness via *in-vitro* studies

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