Structure-Based Design Leads to uPA Loop Mimetic that Accumulates in uPAR-expressing Tumors in Mice

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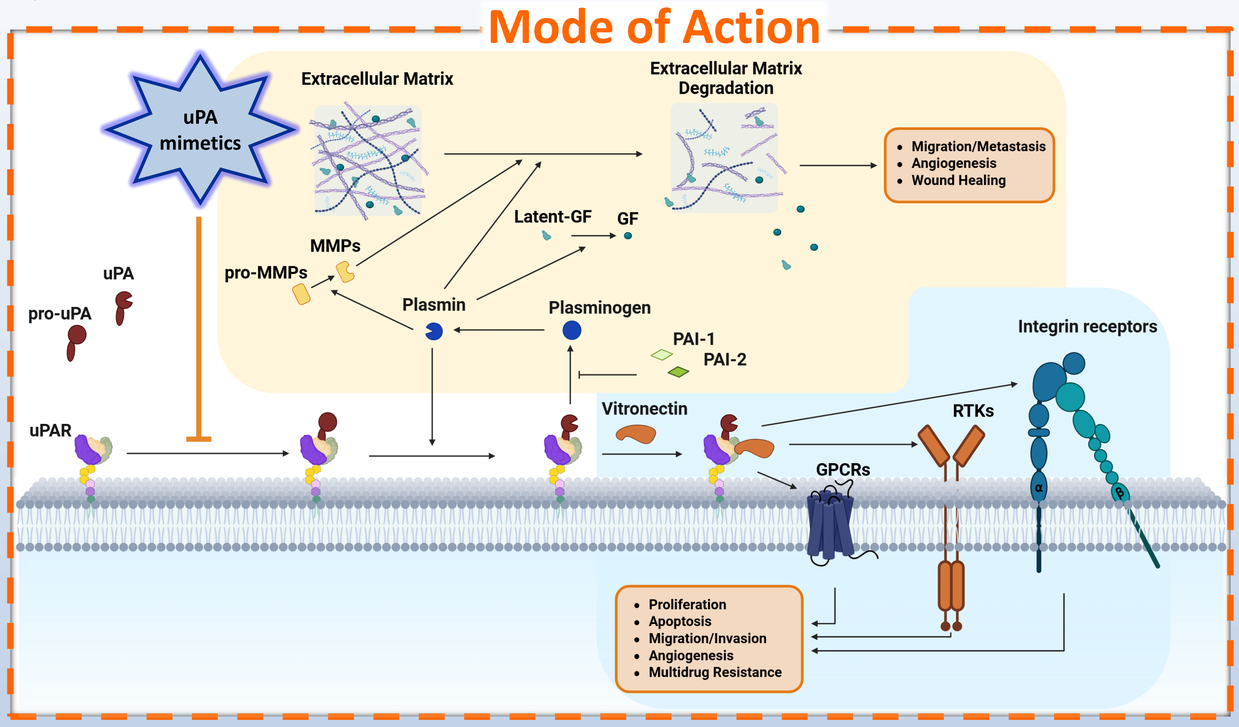
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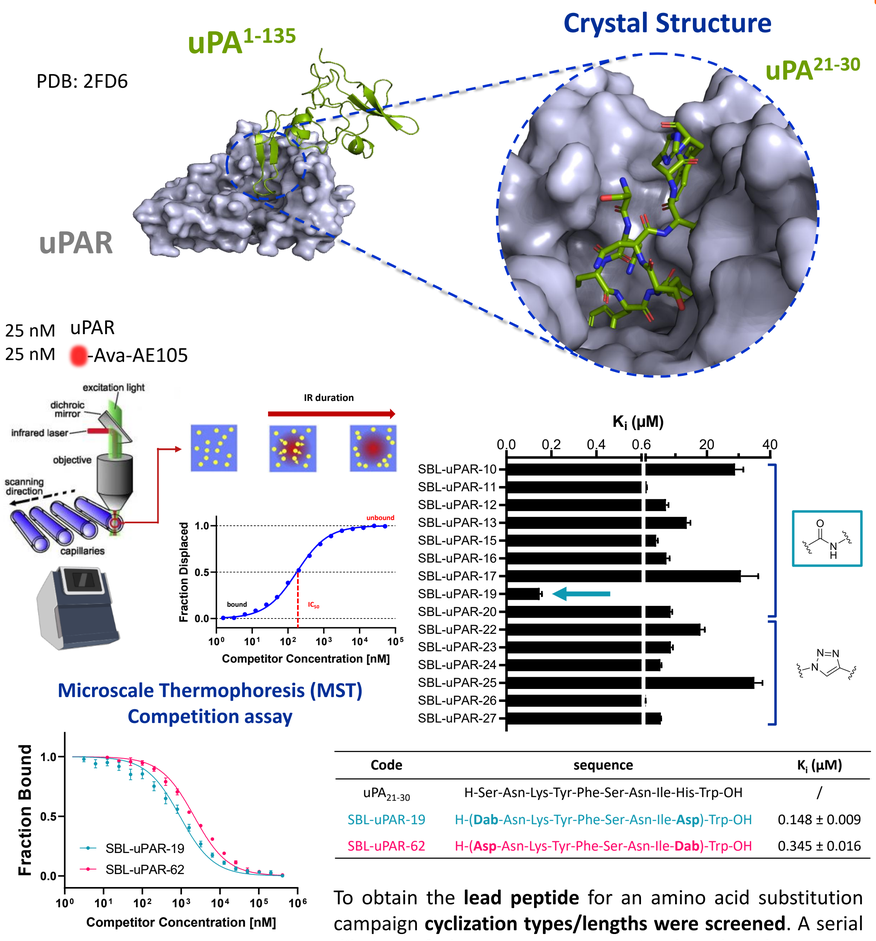
INTRODUCTION

The urokinase-type plasminogen activator receptor (**uPAR**) is a membrane-anchored glycoprotein expressed at low levels during physiological processes such as tissue remodeling and inflammation. However, in many human **cancers** elevated uPAR expression is associated with **poor prognosis**.[1] **In addition to** being a compelling target for **cancer therapy**, uPAR represents a promising **generic imaging target**.[2] Universal uPAR **probes** could **facilitate** the emergence of **fluorescence-guided surgery**, a technique shown to improve tumor-free survival following resective surgery.[3] The loop region uPA$^{21-30}$ of the endogenous ligand uPA interacts within the confined central cavity of uPAR and is responsible for the majority of the binding interactions.[1] Through systematic **structure-based design**, we developed a **high affinity** uPAR **ligand** based on uPA$^{21-30}$, that accumulates in colorectal cancer in mice.

Mode of Action

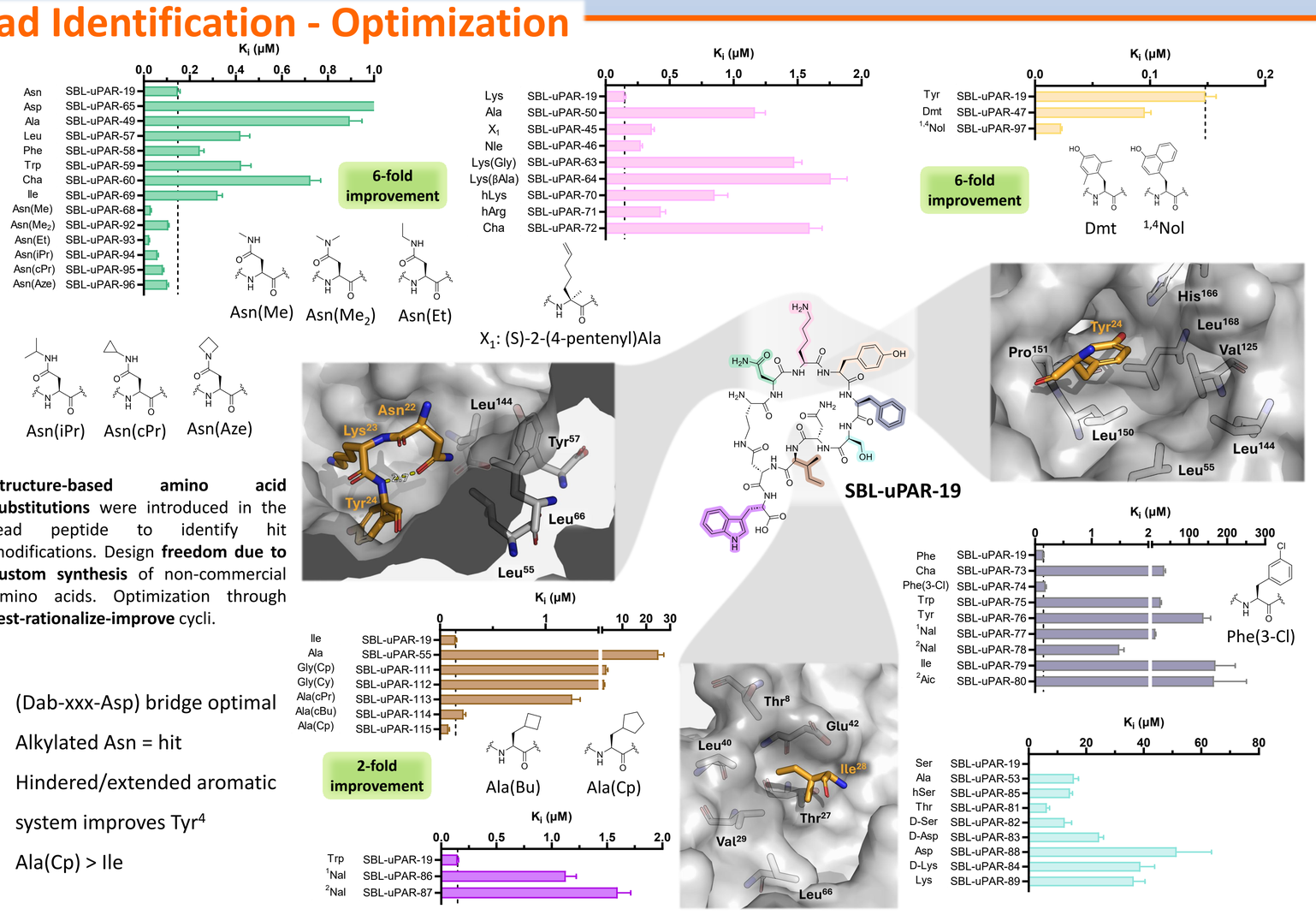


Lead Identification - Optimization



Code	sequence	K_i (μM)
uPA$_{21-30}$	H-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp-OH	/
SBL-uPAR-19	H-(**Dab**-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-**Asp**)-Trp-OH	0.148 ± 0.009
SBL-uPAR-62	H-(**Asp**-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-**Dab**)-Trp-OH	0.345 ± 0.016

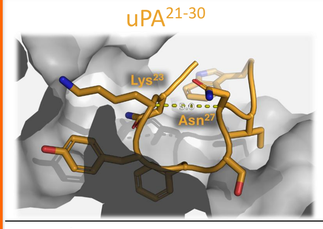
To obtain the **lead peptide** for an amino acid substitution campaign **cyclization types/lengths were screened**. A serial dilution of the competitor was incubated with constant concentration of uPAR and labeled reference peptide AE105. After incubation for 5 minutes, the **thermophoresis** was measured on a Nanotemper Monolith.

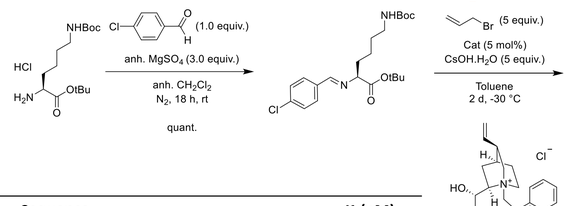


Structure-based amino acid substitutions were introduced in the lead peptide to identify hit modifications. Design **freedom due to custom synthesis** of non-commercial amino acids. Optimization through **test-rationalize-improve** cycli.

- (Dab-xxx-Asp) bridge optimal
- Alkylated Asn = hit
- Hindered/extended aromatic system improves Tyr^4
- Ala(Cp) > Ile

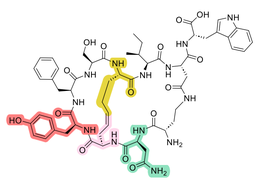
Custom Bicycles – NMR Sructure – High-Affinity Labeled Analogs





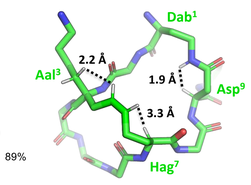
code	Sequence	K_i (nM)
SBL-uPAR-19	Dab Asn Lys Tyr Phe Ser Asn Ile Asp Trp	148 ± 9
SBL-uPAR-50	Ala	1 167 ± 81
SBL-uPAR-66	Dab D-Gly(Allyl) Hag Asp	196 ± 10
SBL-uPAR-91	Dab Aal Hag Asp	11.2 ± 0.3
SBL-uPAR-99	Dab Aal Dmt Hag Asp	0.66 ± 0.06
SBL-uPAR-100	Dab Asn(Et) Aal Hag Asp	0.35 ± 0.04
SBL-uPAR-101	Dab Asn(Et) Aal Dmt Hag Asp	0.17 ± 0.04

Hag: homoallyl glycine

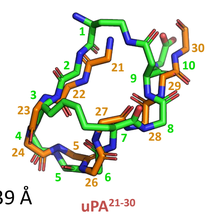


SBL-uPAR-66

NMR Structure



SBL-uPAR-100



SBL-uPAR-110

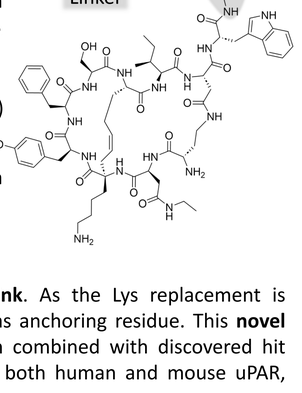
MST direct binding ← TAMRA — K_d = 1.3 ± 0.2 nM (Human); K_d = 0.21 ± 0.15 nM (Mouse)

SBL-uPAR-107

Near-infrared fluorescence imaging ← s775z — K_d = 4.7 ± 0.8 nM (Cells (HT-29))

SBL-uPAR-104

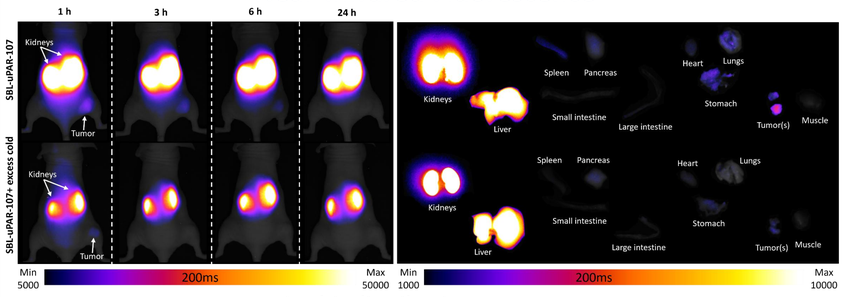
^{111}In chelation SPECT ← DOTA — K_i = 3.3 ± 0.3 nM (Human)



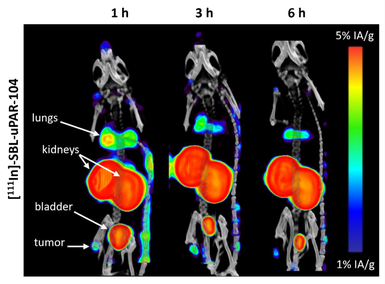
The lead peptide was regidified with a **second crosslink**. As the Lys replacement is detrimental for binding, **Aal was synthesized** and used as anchoring residue. This **novel constrain architecture** resulted in **sub-nM ligands** when combined with discovered hit modifications. The TAMRA-labeled **SBL-uPAR-110** bound both human and mouse uPAR, indicating **species cross-reactivity**.

In Vivo Imaging

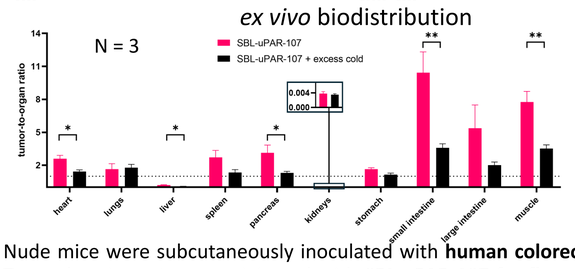
Near-Infrared Fluorescence



SPECT/CT



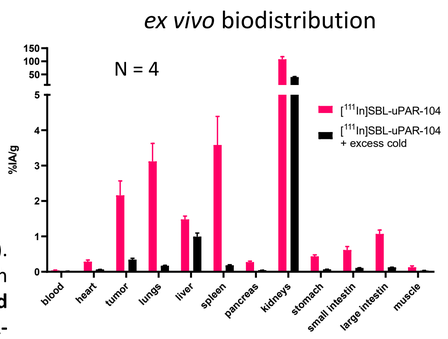
ex vivo biodistribution



dosage
Fluorescence:
SBL-uPAR-107
2 nmol

SPECT:
[^{111}In]**SBL-uPAR-104**
7.45 ± 0.53 MBq
0.5 nmol

ex vivo biodistribution



Nude mice were subcutaneously inoculated with **human colorectal cancer** cells (HT-29). Tumor-bearing mice were injected with **SBL-uPAR-107** for **fluorescence** imaging or with [^{111}In]**SBL-uPAR-104** for **SPECT** imaging, either with or without a 60-fold **excess of cold ligand** (**SBL-uPAR-100**). Images were acquired at 1, 3, 6, 24 h post injection. Clear **uPAR-specific tumoc accumulation** was observed in both imaging modalities.

Conclusions

- ❖ Development sub-nM **uPA mimetic**
- ★ **Approaching K_d uPA** (0.1-0.5 nM)
- ❖ Striking **backbone mimicry**
- ❖ Human-mouse **species cross-reactivity**
- ❖ *In vivo* **imaging contrast** from **1 h p. i.**

Perspective

- ❑ **Combine** hit modifications in **monocycle**
- ❑ Combine ^{1,4}**Nol** & **Ala(Cp)** in **bicycles**
- ❑ Investigate **targeted therapies**
- ❑ **Expand design** for other PPI's

References

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