Mirror-Image Random Nonstandard Peptides Integrated Discovery (MI-RaPID) Technology Yields Highly Stable and Selective Macrocyclic Peptide Inhibitors for Matrix Metallopeptidase 7

https://doi.org/10.17952/38EPS.2026.P325

Hiba Ghareeb*, Choi Yi Li*, Anjana Shenoy, Naama Rotenberg, Julia M. Shifman, Takayuki Katoh, Irit Sagi, Hiroaki Suga, Norman Metanis

Institute of Chemistry, Hebrew University of Jerusalem · University of Tokyo · Weizmann Institute of Science

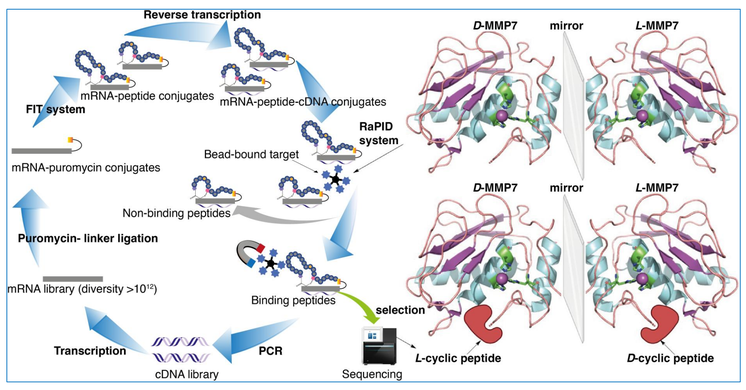
BACKGROUND & RATIONALE

MMP7 (matrilysin) is the smallest MMP, overexpressed in colorectal, gastric, pancreatic and lung cancers and associated with tumor metastasis and poor survival.

Key challenge: Conservation of active sites across 23 MMPs makes selective inhibition very difficult.

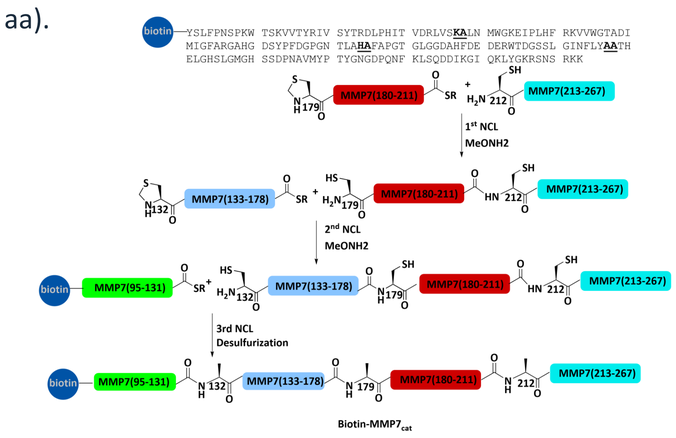
Existing MMP7 inhibitors: moderate selectivity, limited stability — no viable clinical candidate.

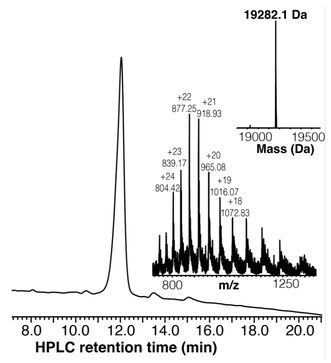
Our approach: Exploit *D*-amino acid chemistry to create innately protease-resistant macrocyclic peptide inhibitors with high selectivity.

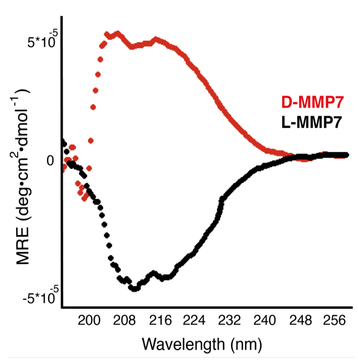


CHEMICAL SYNTHESIS OF L- and *D*-MMP7

Chemically synthesize MMP7 (mirror-image protein) via Native Chemical Ligation + desulfurization (4 segments, 173 aa).





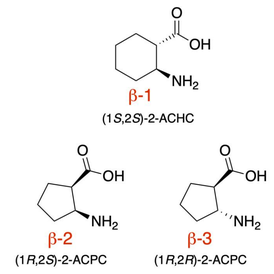


SELECTION & HIT ID

6 rounds of RaPID affinity selection against both *L*- and *D*-MMP7 immobilized on streptavidin-coated magnetic beads.

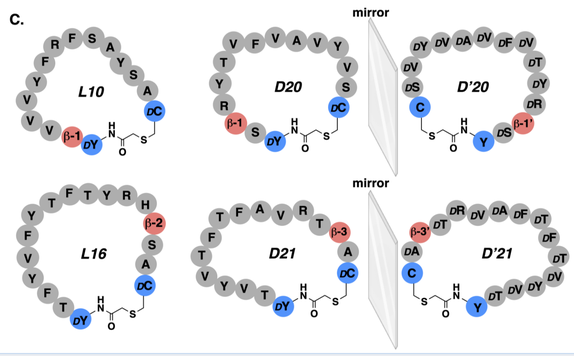
a.

1st	2nd U	2nd C	2nd A	2nd G	3rd
U	Phe	Ser	Tyr	β-3	U
	Leu	Ser	Stop	D-Cys	C
					A
					G
C	β-2	Pro	His	Arg	U
	Leu	Pro		Arg	C
					A
					G
A	β-1	Thr	Asn	Ser	U
	ClAcD-Tyr	Thr	Lys	Arg	C
					A
					G
G	Val	Ala	Asp	Gly	U
	Val	Ala		Gly	C
					A
					G



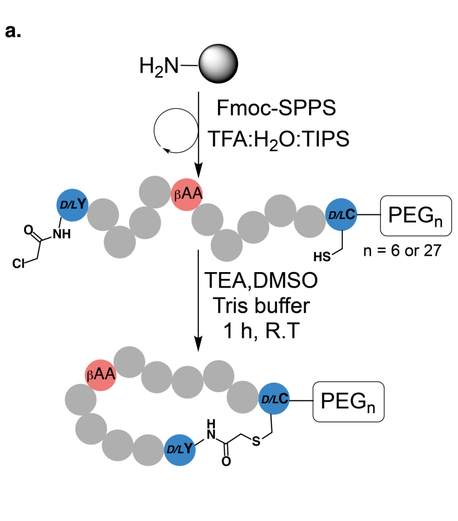
Top hits selected for synthesis:

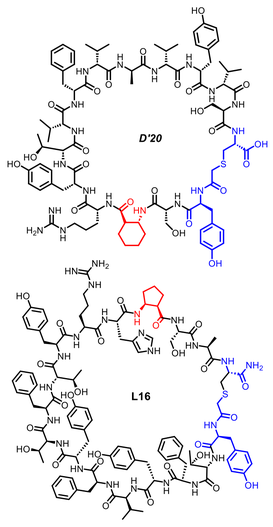
- L10, L16 (L-MMP7 selection)
- D20, D21 (D-MMP7 selection) → mirror-image forms *D'20, D'21*



LEAD COMPOUND SYNTHESIS

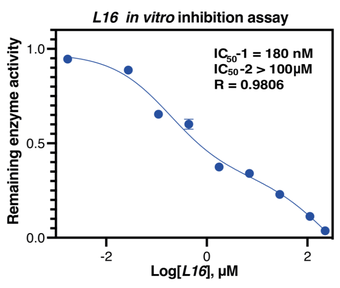
a.



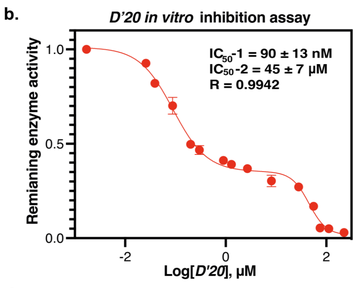


LEAD COMPOUND: *D'20*

IC_{50} = 90 nM vs MMP7

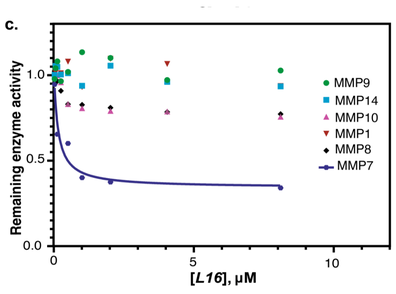


b.

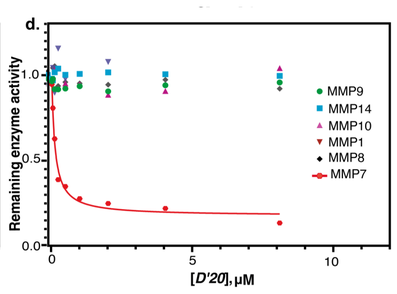


D`20 >60× selectivity over MMP1/8/9/10/14

c.



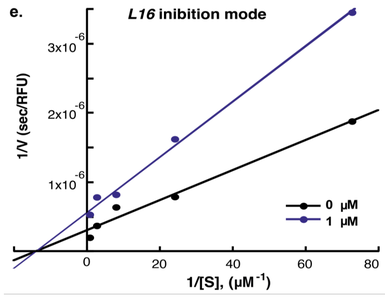
d.



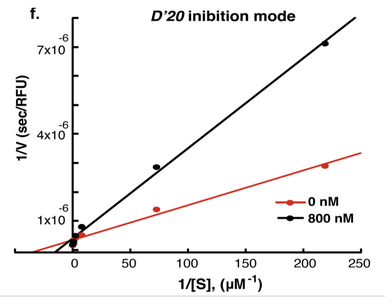
LEAD COMPOUND: *D'20*

Competitive inhibition mode

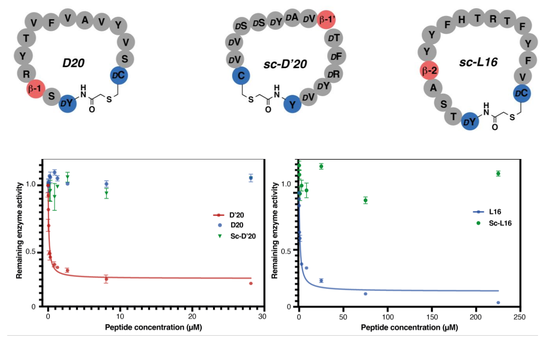
e.



f.



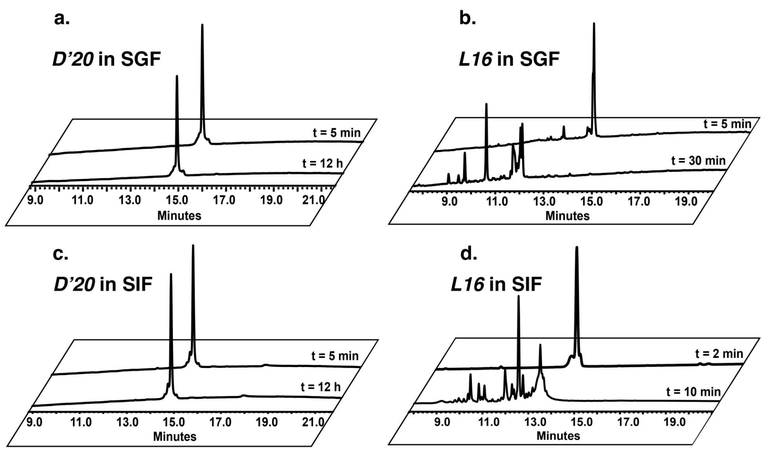
Sequence and Stereospecific



STABILITY — SERUM & GI

Human serum: Both *D'20* and L16 are stable for >24 h.

Condition	*D'20*	L16
Simulated Gastric Fluid (SGF, pH 1.3, pepsin)	>12 h ✓	<30 min ✗
Simulated Intestinal Fluid (SIF, pH 6.8, pancreatin)	>12 h ✓	<10 min ✗



CELL MIGRATION INHIBITION

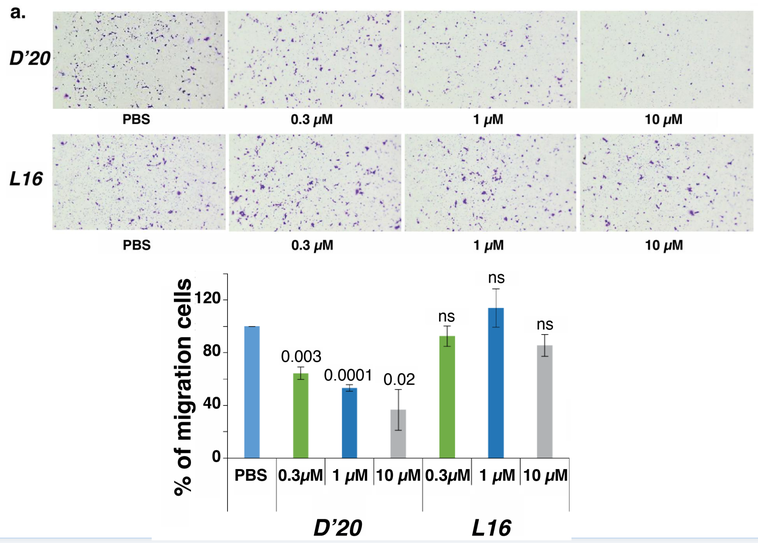
Pancreatic cancer cell line CFPAC-1 (endogenous MMP7 expression). Transwell migration assay with FBS chemoattractant (18 h).

D'20 migration IC_{50} ≈ 1 μM

Comparable to GSM-192, a highly selective anti-MMP7 monoclonal antibody (IC_{50} = 132 nM enzymatic inhibition).

No effect on cell proliferation or viability at tested concentrations — *D'20* specifically targets migration.

a.



CONCLUSIONS

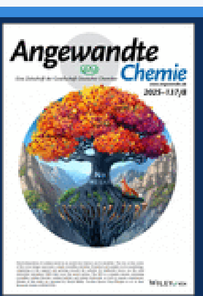
▶ MI-RaPID enables de novo discovery of fully D-amino acid macrocyclic inhibitors — bypassing the D-amino acid incorporation limit of standard RaPID.

▶ *D'20* is potent (IC_{50} 90 nM), selective (>60× over related MMPs), stereospecific, and sequence-specific.

▶ Exceptional stability in human serum and GI fluids (>12 h) makes *D'20,* a key advantage over L-peptide comparators.

▶ *D'20* inhibits pancreatic cancer cell migration (IC_{50} ~1 μM) without affecting proliferation or viability.

▶ MI-RaPID is a broadly applicable platform — not limited to MMPs — for any overexpressed disease target.



Ghareeb, Hiba, Choi Yi Li, Anjana Shenoy, Naama Rotenberg, Julia M. Shifman, Takayuki Katoh, Irit Sagi, Hiroaki Suga, and Norman Metanis. "Mirror-image random nonstandard peptides integrated discovery (MI-RaPID) technology yields highly stable and selective macrocyclic peptide inhibitors for matrix metallopeptidase 7." *Angewandte Chemie* 137, no. 8 (2025): e202414256.

PCT/IL2025/050321

Dreaming. Creating. Innovating. Since 1925

THE HEBREW UNIVERSITY OF JERUSALEM