

# **Combinatorial Engineering of Proteolytically Resistant APPI Variants that Inhibit Human Mesotrypsin for Prostate Cancer Therapy**

Itay Cohen<sup>1</sup>, Evette Radisky<sup>2</sup> and **Niv Papo**<sup>1</sup>

<sup>1</sup> Department of Biotechnology Engineering and National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel

<sup>2</sup> Department of Cancer Biology, Mayo Clinic Cancer Center, Florida, USA

**Background** - Mesotrypsin, an enzyme that contributes to progression and metastasis of prostate cancer, constitutes a compelling therapeutic target. However, with its unique capability for cleavage and inactivation of proteinaceous inhibitors, mesotrypsin presents a formidable challenge to the development of biologic inhibitors.

**Methods** - Our study identifies a promising mesotrypsin inhibitor – a triple mutant of the human amyloid precursor protein Kunitz protease inhibitor domain (APPI) with superior affinity, specificity, and proteolytic stability – as a starting point for the development of anticancer protein therapeutics.

**Results** - We demonstrate that the mutant acts as a functional inhibitor of mesotrypsin-dependent prostate cancer cellular invasiveness. Additionally, the crystal structure of the mutant/mesotrypsin complex provides new insights into the structural and mechanistic basis for the mutant's improved binding and proteolytic resistance. The antagonistic activity of the selected mutant is currently being tested in *in-vivo* models of prostate cancer.

**Conclusions** - The study establishes proof-of-principle for a novel library screening approach that is widely applicable for simultaneously evolving proteolytic stability and a desired functionality for diverse protein scaffolds.

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