Engineered Peptide-Based Nanosystems for Improving Therapeutic and Diagnostic Potential of Anticancer agents

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PEPTIDES : • *High specificity* • *Low toxicity* • *Low immunogenicity*

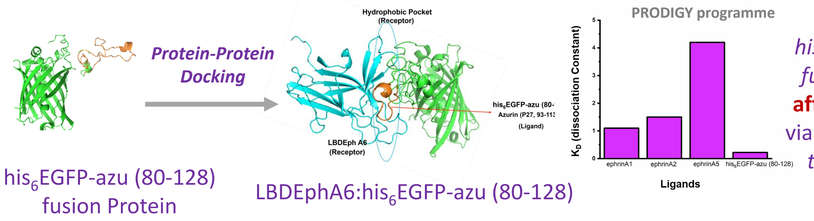
ENGINEERED PROTEINS & PEPTIDES TRANSFORMED INTO MULTIFUNCTIONAL NANOMEDICINES

FROM MOLECULAR DESIGN TO CANCER RESPONSE

DESIGN → SELF-ASSEMBLE → TARGET → IMAGE → TREAT

NANOSYSTEM : his$_6$EGFP-azu (80-128 aa)

(A). BIOMARKER CELL SURFACE RECEPTOR, EPHA6 RECOGNITION BY ENGINEERED his$_6$EGFP-AZU(80–128): AN *IN-SILICO* TARGETING STRATEGY

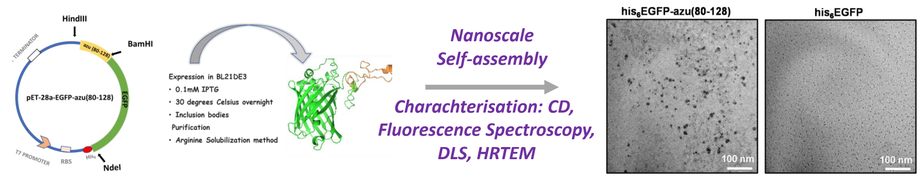


his$_6$EGFP-azu (80-128) fusion Protein

LBDEphA6:his$_6$EGFP-azu (80-128)

his$_6$EGFP-azu(80–128) fusion, showed **high-affinity** EphA6 binding via azu(80–128) *than its traditional ephrin A ligands.*

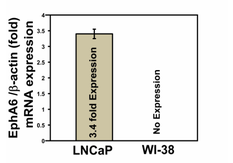
(B). his$_6$EGFP-azu(80–128) NANOSYSTEM ENGINEERING



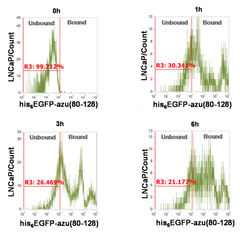
his$_6$EGFP-azu(80–128) engineered and purified as a recombinant fusion protein

HRTEM Characterisation :**~20–25 nm Spherical Nanoparticles**

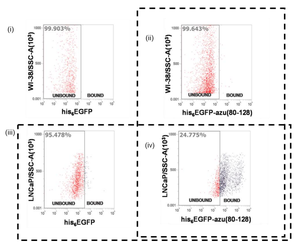
(C). SELECTIVE CANCER TARGETING (EphA6-DEPENDENT CELLULAR BINDING)



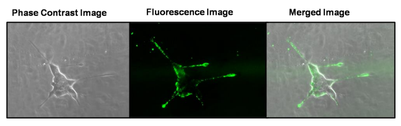
LNCaP: **~3.4-fold** EphA6 mRNA expression
WI-38: No detectable EphA6 expression



his$_6$EGFP-azu(80-128) binds **~70%** cell population within 1 hour

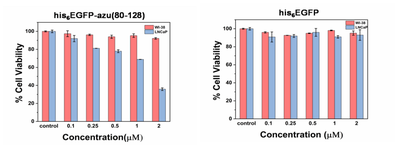


his$_6$EGFP-azu(80-128) drives selective EphA6$^+$ cancer-cell targeting
EphA6$^-$ → ~0.4%
EphA6$^+$ → ~76%

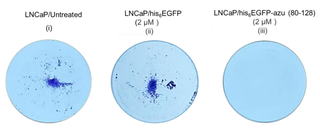


Fluorescent his$_6$EGFP-azu(80-128) Nanosystem enables **Cancer-cell Imaging**

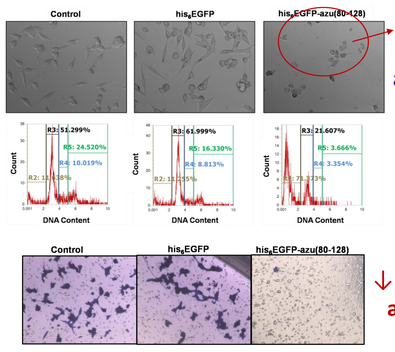
D). CYTOTOXICITY, ANTI-METASTATIC, ANTI-PROLIFERATIVE ACTIVITY AGAINST LNCaP CELLS.



MTT/Overnight: >50% Cancer-cell Killing at **2 μM** his$_6$EGFP-azu(80–128)



15-day **COLONY FORMATION ASSAY**: **Complete suppression** of clonogenic growth



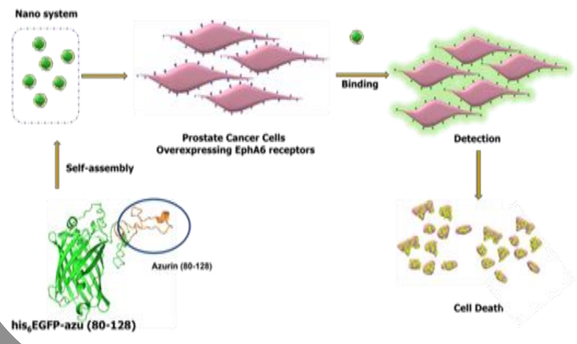
Loss of normal cell morphology and reduced cell density

CELL-CYCLE ANALYSIS
↓ S-phase population
↓ G_2/M population
altered cell-cycle progression

TRANS-WELL ASSAY: reduced cell migration, reduced cell invasion

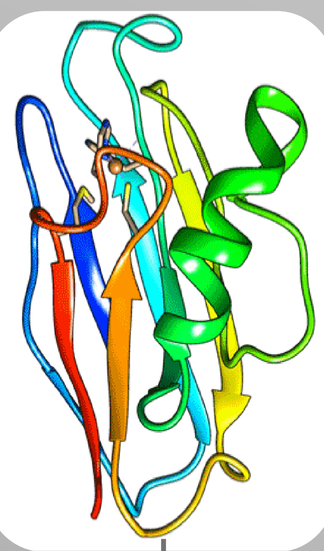
KEY FINDINGS

✓ *Engineered azurin converts a naturally bioactive protein into a multifunctional* ***theranostic nanosystem*** *for selective cancer targeting and therapy.*



✓ **SELF-ASSEMBLES** → *~20–25 nm nanosystem*
✓ **TARGETS** → *EphA6$^+$ prostate cancer cells*
✓ **IMAGES** → *EGFP-enabled cancer-cell visualization*
✓ **KILLS** → *>50% cancer-cell killing at 2 μM*
✓ **SUPPRESSES** → *Migration, invasion & clonogenic growth*

AZURIN



WHY AZURIN?

NATURAL ANTICANCER PROTEIN
128 aa • ~14 kDa • Cu-containing redox protein
From Pseudomonas aeruginosa

CANCER-SELECTIVE ACTIVITY
Preferential cancer-cell entry → selective cytotoxicity

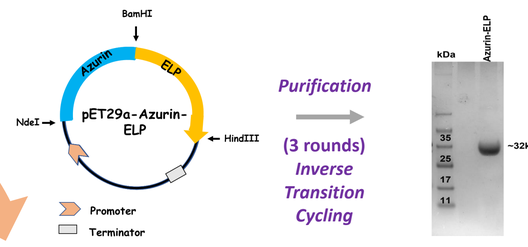
ANTICANCER HOTSPOTS
P28 (azu 50–77 aa) + C-terminal region
→ Key regions linked to anticancer activity

MULTIPLE CANCER PATHWAYS
Azurin interacts with cancer-associated signaling pathways
→ Growth • migration • invasion

TARGETING BEHAVIOUR
C-terminal region
Eph receptors → cancer progression & metastasis
→ A rationale for EphA6-targeted nanomedicine

NANOSYSTEM : Azurin-(Elastin-like Polypeptide)

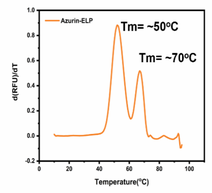
(A). CLONING, EXPRESSION, AND PURIFICATION OF RECOMBINANT FUSION PROTEIN AZURIN-ELP DI-BLOCK COPOLYMER



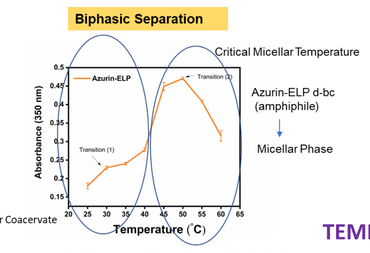
Recombinant Plasmid vector containing fused genes, *AZURIN* and *ELP*

STRUCTURE RETAINED → *Azurin maintains its structural integrity post-ELP fusion.*
CYTOTOXICITY PRESERVED → *Anticancer activity remains intact in the conjugate.*
CELLULAR UPTAKE MAINTAINED → *Efficient cancer-cell internalization is retained.*

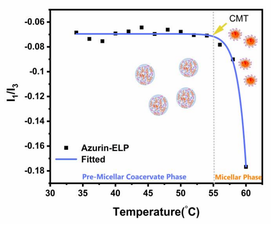
(B). TEMPERATURE-TRIGGERED SELF-ASSEMBLY OF AZURIN-ELP D-BC



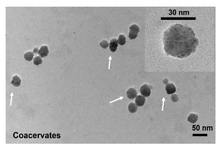
THERMAL SHIFT ASSAY : Phase Transition temperatures



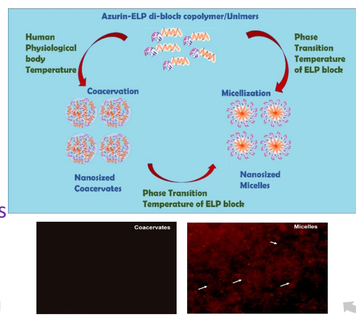
UV-VISIBLE SPECTROSCOPY: showing Biphasic Separation

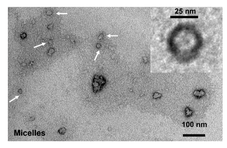


TEMPERATURE-TRIGGERED MICELLIZATION
Pyrene fluorescence (I_1/I_3) → Critical micellization temperature → MICELLE FORMATION

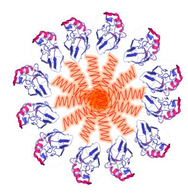


HRTEM :Coacervates (Size: ~ 30 nm)





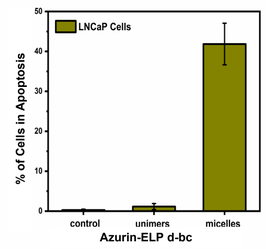
HRTEM :Micelles (~ Size: 25 nm)



THERMORESPONSIVE TRANSFORMATION
Coacervates (~37-40 °C) → Micelles (~50-55 °C)
Potential platform for controlled drug & contrast-agent delivery

SYPRO ORANGE FLUORESCENCE visualizes temperature-driven morphological transformation

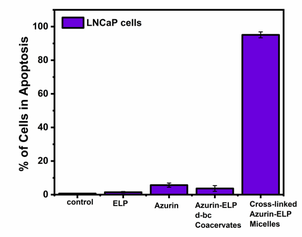
(C). EVALUATION OF CYTOTOXICITY OF THE MICELLAR NANOSTRUCTURES OF AZURIN-ELP D-BC



UNIMERS VS MICELLES : **~ 38%** Cancer cells in Apototic Phase at 5 μM concentration

THERAPEUTIC EFFICACY ?

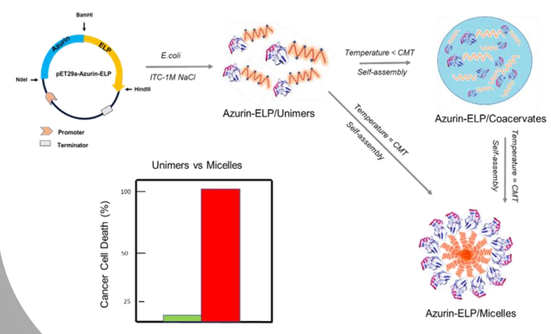
The crosslinked azurin-ELP micelles were more effective.



COMPARATIVE CYTOTOXICITY: **~ 97%** Cancer cells in Apototic Phase at 20 μM concentration

KEY FINDINGS

✓ *First study to examine utility of ELP based nano-delivery of azurin.*



✓ **THERMORESPONSIVE SELF-ASSEMBLY**
→ *Programmable temperature-triggered nanostructure formation*
✓ **NANOSCALE ORGANIZATION**
→ *~25–30 nm micelles*
✓ **ENHANCED CYTOTOXICITY**
→ *Improved anticancer activity against cancer cells by micelles*
✓ **THERAPEUTIC DELIVERY**
→ *A modular platform for cargo encapsulation and delivery*

TAKE-AWAYS

Engineering **peptides** into **multifunctional nanosystems** established a modular platform for precision oncology, integrating:

CANCER-CELL TARGETING

FLUORESCENCE IMAGING

THERMORESPONSIVE SELF-ASSEMBLY

THERAPEUTIC DELIVER

PRECISION NANOMEDICINE

References:
1. R. Bhardwaj and P Mishra, *Molecular Pharmaceutics* **2023**, *20*, 6066–6078.
2. R. Bhardwaj and P Mishra, *Biomacromolecules* **2024,** *25*, 508-521.