Conformational and self-assembly studies of a protein-mimetic peptide from *Pseudomonas aeruginosa*'s YeaZ protein

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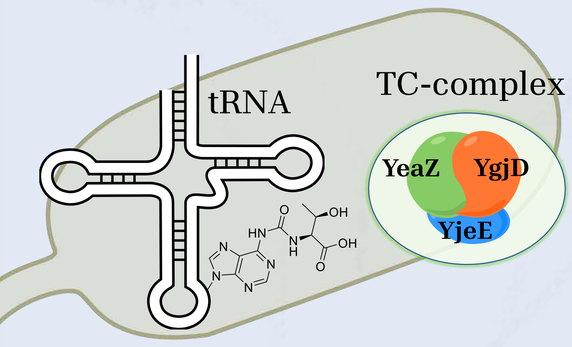
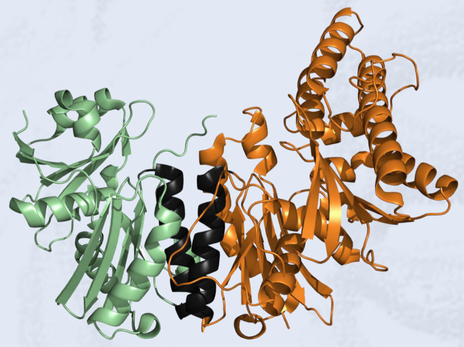
BACKGROUND





YeaZ-YgjD complex (6z81)

Protein-mimetic peptides (PMPs):
shorter sequences of proteins and biocompatible building blocks with multiple applications.

Self-assembly:
association process of individual building block units into highly ordered structures.

PaYeaZ:
self-assembling protein from *P. aeruginosa*, involved in the formation of an essential protein complex.

PaYeaZ shows protein-protein interactions thanks to an α-helical surface domain → **Study of aggregation properties of the PMP for material science applications**

STUDY

Peptides' design

- Identification of the shortest sequence mainly responsible of **protein-protein interactions** in YeaZ homodimer
- Determination of the essential residues for **interactions and helicity**

METHODS

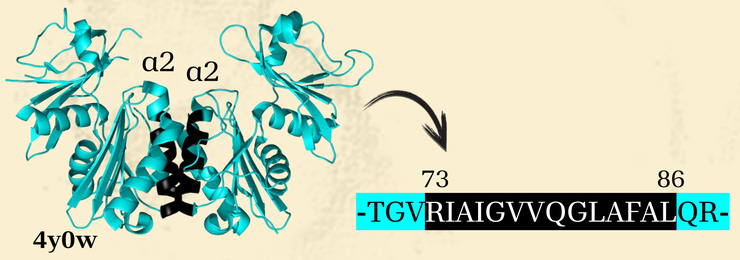
Computational studies

- Identification of the **surface accessible residues** in the homodimer of YeaZ (4y0w)

Vecchietti, Davide, *et al. BBRC* 470.2 (**2016**): 460-465

- **Hot spots** identification by Computational Alanine Scanning (CAS)
- Introduction of a **polar group** at the C-terminal to enhance hydrophilicity and aggregation properties

RESULTS



Arg73 – Leu86 in the YeaZ protein is the main responsible domain for protein-protein interactions

First Subhunit		Second Subhunit	
Mutation	ΔΔG (kcal/mol)	Mutation	ΔΔG (kcal/mol)
THR70ALA	-0.5497	THR70ALA	0.3585
VAL72ALA	0.0956	VAL72ALA	0.0717
ARG73ALA	-13.0972	ARG73ALA	-8.6279
ILE74ALA	3.5133	ILE74ALA	0.6214
ILE76ALA	0.2629	ILE76ALA	0.8843
VAL78ALA	2.1032	VAL78ALA	1.5774
GLN80ALA	-0.1673	GLN80ALA	-1.434
LEU82ALA	3.2982	LEU82ALA	2.2705
PHE84ALA	7.5524	PHE84ALA	10.6594
LEU86ALA	1.6491	LEU86ALA	1.3862

ΔΔG < 0 : more stable with Ala
ΔΔG > 0 : less stable with Ala

- **Ile74, Val78, Leu82, Phe84**: hot spots
- **Arg73**: substituted with Ala

AIAIGVVQGLAFAL **PMP1**

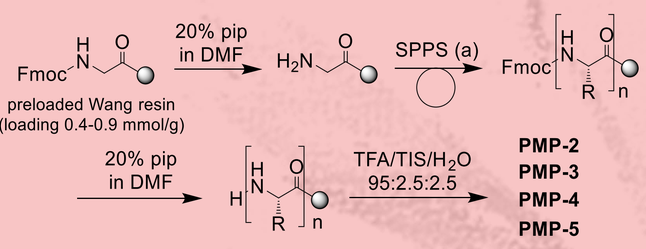
↓

AIAIGVVQGLAFALGGKKKG **PMP2**

Peptides' synthesis and characterization

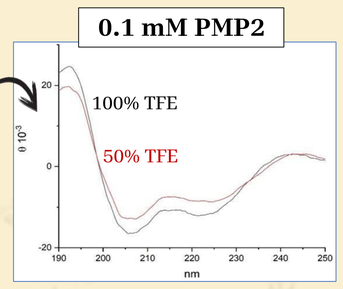
- **PMP2** and shorter derivatives **PMP3-5**
- Secondary structure by Circular Dichroism

Solid Phase Peptide Synthesis



[a] Coupling conditions – 1: Fmoc-aa-OH 3-5 eq, DIPEA 9-15 eq, COMU 3-5 eq. Coupling conditions – 2 : Fmoc-aa-OH 3-5 eq, DIC 3-5 eq, Oxyma 3-5 eq.

Name	Sequence	Yield	Purity	Helix%
PMP-2	AIAIGVVQGLAFALGGKKKG	60%	96%	35%
PMP-3	VVQGLAFALGGKKKG	42%	97%	7%
PMP-4	LAFALGGKKKG	25%	95%	8%
PMP-5	AIAIGVVQGGKKKG	36%	98%	12%

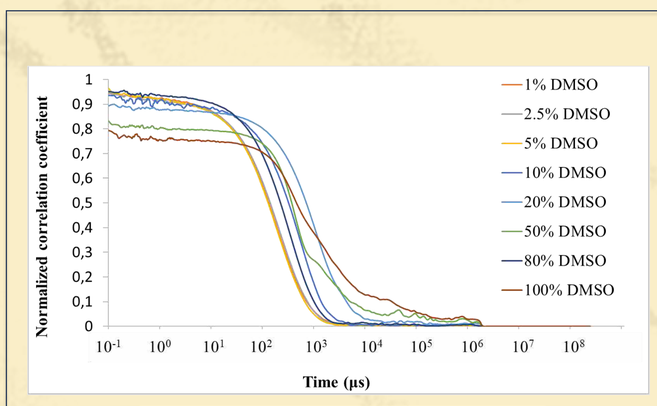


Only **PMP2** is α-helical and able to form supramolecular aggregates.

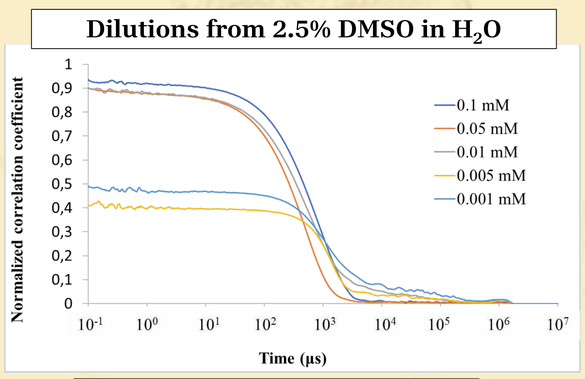
Supramolecular aggregates morphology studies

- Comparison between hydrophilic and hydrophobic environment
- Dependance on concentration
- Aggregates stability in DMSO

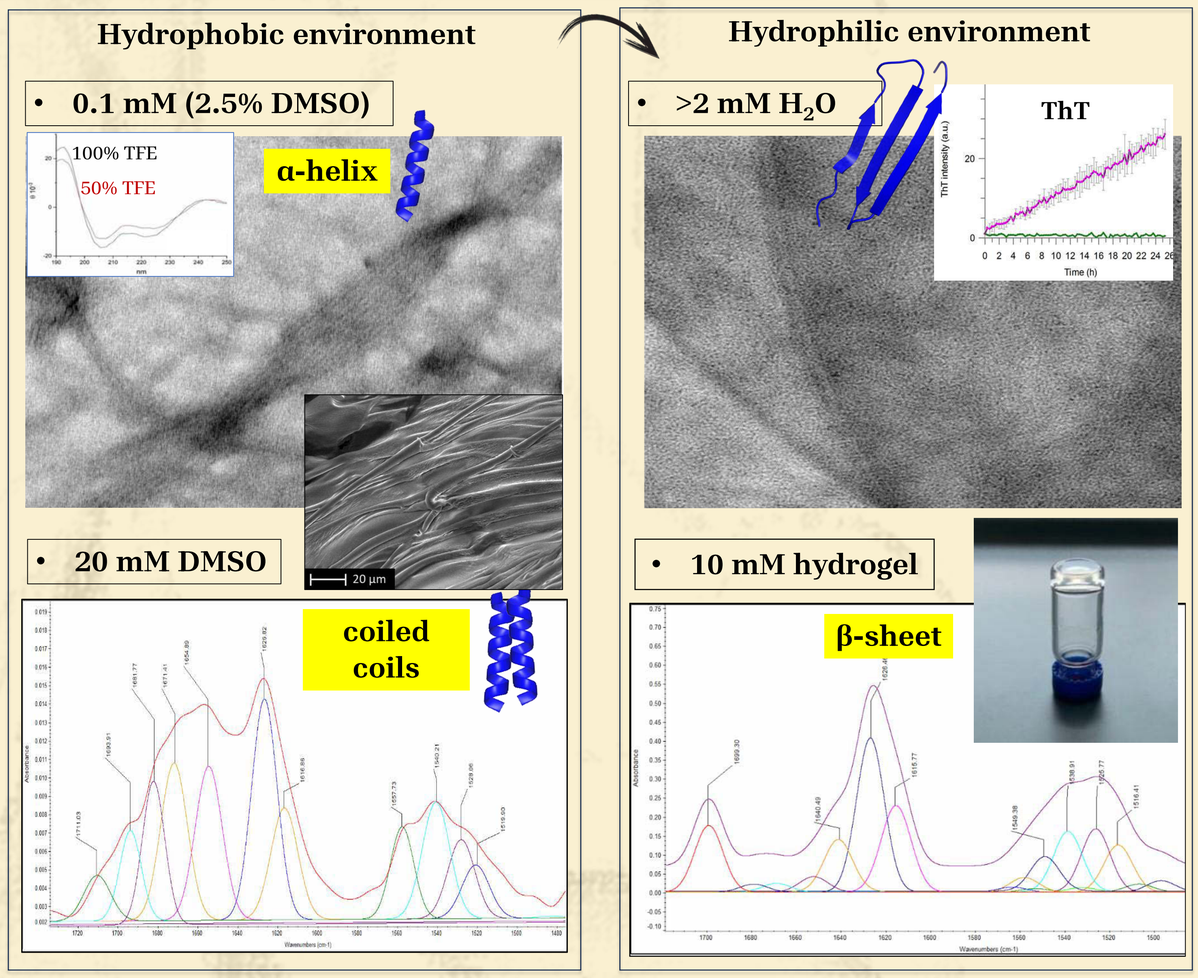
- **Scanning and Transmission Electron Microscopy (solvent displacement DMSO to water)**
- **Thioflavin T assay**
- **Dynamic Light Scattering**
- **Attenuated Total Reflectance**



- **PMP2** is stable to all DMSO conditions



- Stable aggregates are formed above 0.01 mM



Fasola, Elettra, *et al.*, *Front. Chem.* (**2022**) 10:1038796

OUTLOOK AND CONCLUSION

- **PMP1** is the main sequence responsible for YeaZ-YeaZ and YeaZ/YgjD interactions
- **PMP2** is an amphiphilic, more soluble derivative of **PMP1**, and the shortest sequence able to maintain helicity outside the protein environment
- The secondary structure is related to aggregation abilities: only the helical **PMP2** forms aggregates
- The secondary structure and type of aggregates depends on the environment (hydrophobic vs hydrophilic)
- **PMP2** aggregates are stable to DMSO, and in water a stable hydrogel is formed

Overall, we were able to obtain a biocompatible protein-mimetic building block, able to maintain α-helix and to form aggregates. A conformational switch to β-sheet occurs in hydrophilic conditions.