A structural sight on peptides forming stable LLPS: a combined experimental and computational investigation

Gianfranco Bocchinfuso[1], Francesca Cavalieri[1] , Daniel O. Cicero[1], Annachiara Ferrara[1], Hilda Garay-Perez[2], Greta Petrella[1], Marco Savioli[1], Mariano Venanzi[1]

[1]Department of Chemical Sciences and Technology, University of Rome Tor Vergata, 00133, Rome, Italy;
[2]Centre for Genetic Engineering and Biotechnology, PO Box 6162, 10600, Havana, Cuba;
https://doi.org/10.17952/37EPS.2024.P1320

PAS peptides

- PAS-based polypeptides are recombinant or synthetic peptides
- Typically, PASs lack any secondary structure
- Recently proposed as an alternative to polyethylene glycol (PEG)
- Used as components of nanoparticles for different applications

Breibeck J. and Skerra A. Biopolymers. 2018, 109:e23069

Liquid Liquid Phase Separation (LLPS)

- Important in the organization and function of cellular components
- Considered for designing new biomolecular dynamic condensates
- Described also for peptides
- Little studied in peptides lacking charges or aromatic residues

Brangwynne, C. P. et al. Science 2009, 324:1729 Leshem, B. et al. Nature Communications 2023; 14:421

Investigated systems

PAS sequences containing short repeating sequences.

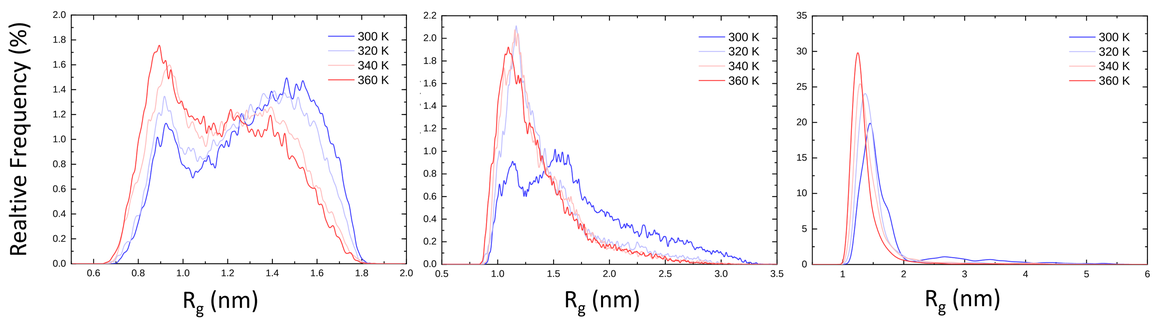
PAS20 : (PASPAAPAPSAPAASPAAPA)–COOH
PAS40: $(PASPAAPAPSAPAASPAAPA)_2$–COOH
PAS80: $(PASPAAPAPSAPAASPAAPA)_4$–COOH

Possibility to investigate the effect of chain length.

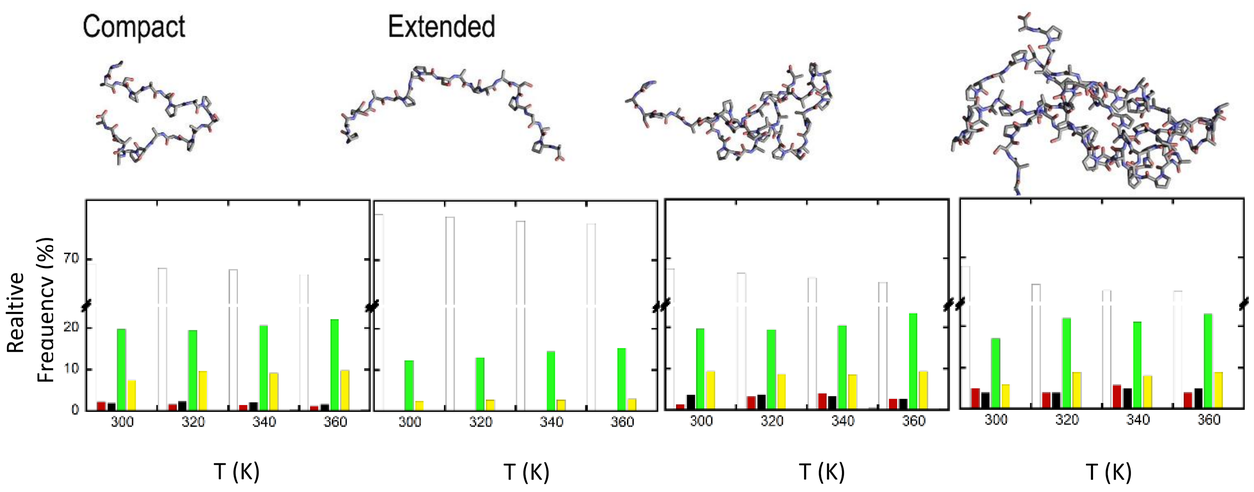
Monomers populate two conformations

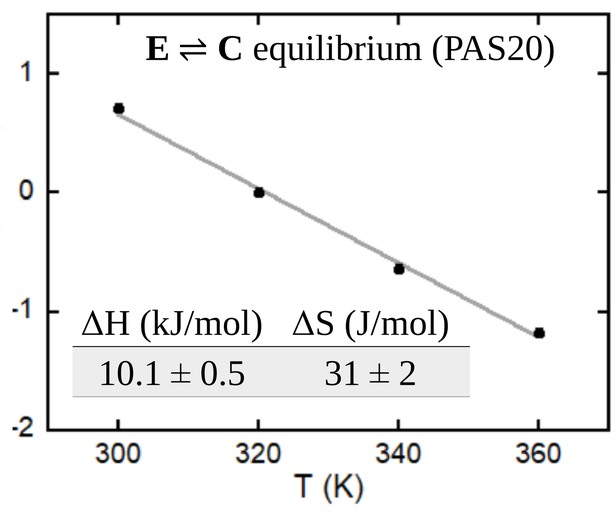
Molecular Dynamics Simulations at different temperatures

Gyration Radius (Rg)



Secondary Structure (Coil B-sheet B-Bridge Bend Turn 3-Helix)

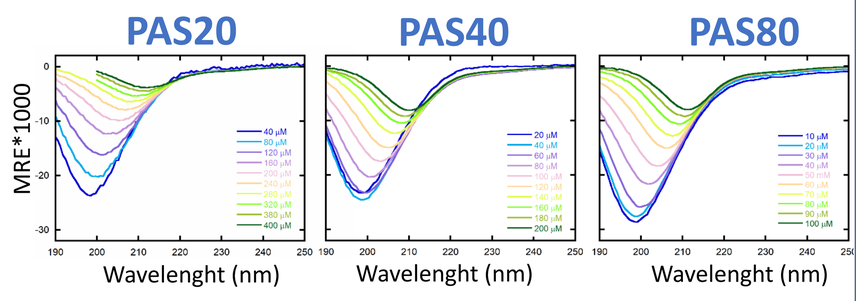




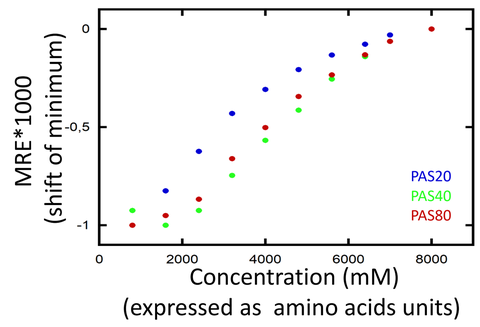
- An equilibrium between extended (**E**) and compact (**C**) conformations exists
- **C** are favored at higher T and high MW
- **C** are characterized by the presence of β motifs
- The equilibrium toward **C** is entropically driven
- CD spectra (not shown) at low concentrations confirm MD results

PASs exhibit high mobility in the aggregates (LLPS)

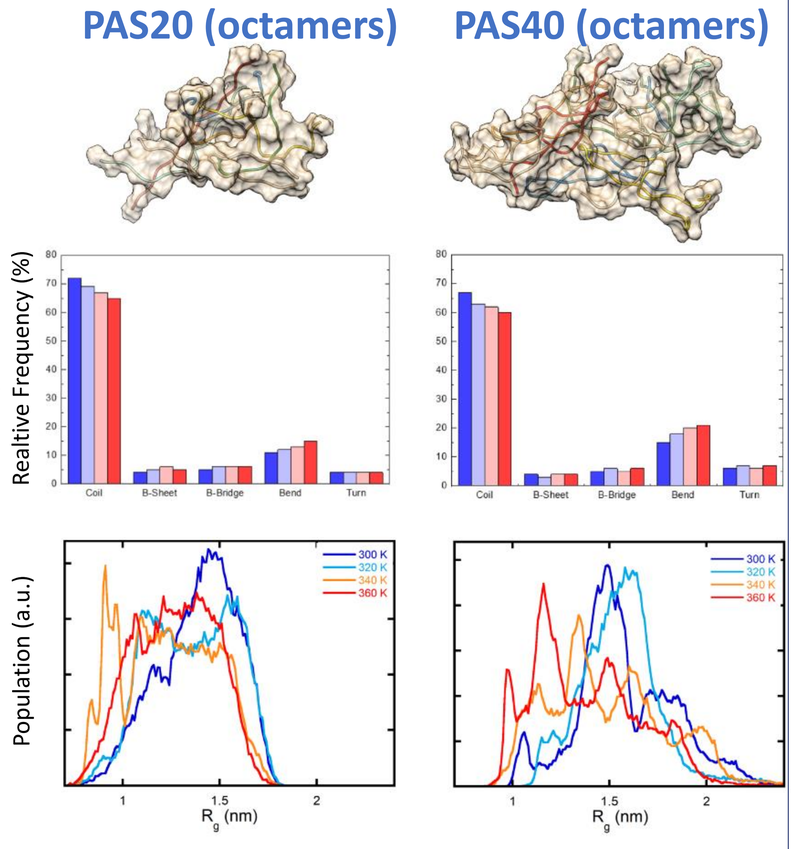
Changing in the **CD spectra** with concentration is due to PAS aggregation.



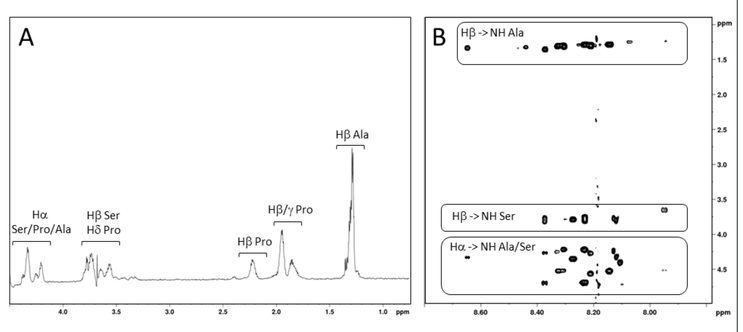
Aggregation depends on the concentration of amino acid units in the solution.



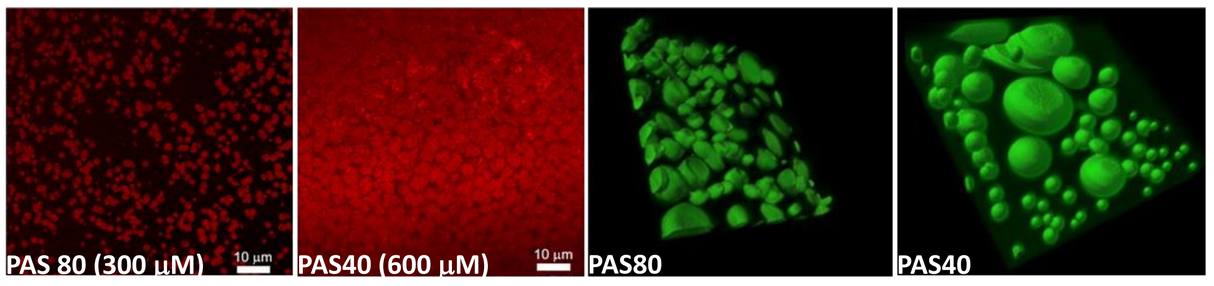
MD simulations of systems containing 8 replicas of PAS20 and PAS40 show **high flexibility** of the peptides in the aggregates. Peptides in the aggregates populate preferentially compact conformations.



NMR data of PAS80 at high concentrations confirm the **extreme mobility** of peptides in the aggregates



Confocal microscopy images with 3D reconstruction of FITC labeled PAS show the formation of condensates 3-5 microns in size



Conclusions

- Under the C.A.C., an equilibrium between extended and compact conformations exists
- PAS peptides aggregate at critical concentrations of amino acid units in the solution, almost independently by the chain length
- PAS sequences remain extremely flexible in the aggregate and the aggregates form condensates 3-5 microns in size.

Despite the absence of charges and aromatic rings, the investigated PAS sequences show LLPS

PEPSA-MATE
Nanopeptides and Nanosaccharides for Advanced and Sustainable Materials
CINECA
PRACE