EVOLUTIONARILY CONSERVED AMYLOID ARCHITECTURE IN THE PACAP–GLUCAGON PEPTIDE SUPERFAMILY

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AIM OF THE STUDY

To characterize the structural basis of amyloid formation within the human PACAP–glucagon peptide hormone superfamily.

WHY IS THIS IMPORTANT?

Members of the human PACAP–glucagon peptide hormone superfamily act through class B G protein-coupled receptors (GPCRs) to regulate diverse metabolic, neural, endocrine, and developmental functions. Their synthetic analogues have major therapeutic potential, exemplified by the current rise of GLP-1-based therapies.

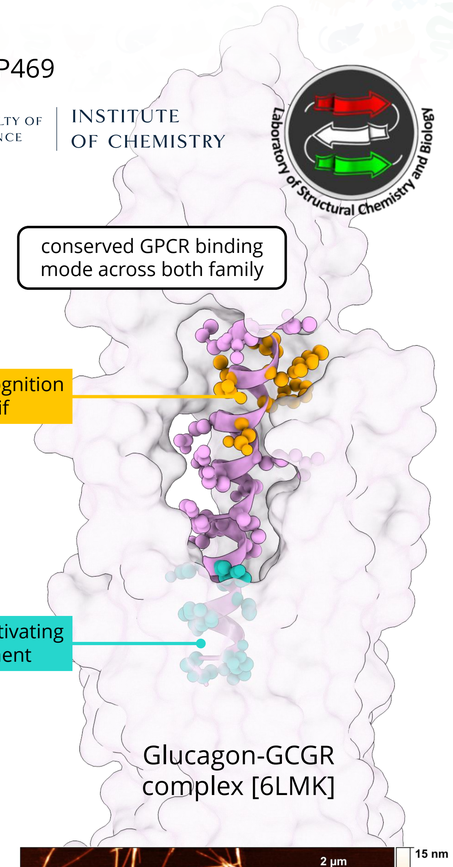
However, their efficient synthesis and formulation are challenged by their intrinsic aggregation propensity, which is linked to their physiological storage as amyloid assemblies (R1) at high concentrations in acidic secretory vesicles (R2) prior to release into the bloodstream.

Unlike pathological amyloids, these assemblies represent a functional and reversible state. Understanding their structural basis may therefore provide a basis for novel peptide formulation strategies.

GCG – glucagon; **GLP-1/2** - glucagon-like peptide-1/2; **GIP** - glucose-dependent insulinotropic polypeptide; **PACAP** - pituitary adenylate cyclase activating polypeptide; **PRP** – PACAP-related polypeptide; SCT – secretin; **GHRH** - growth hormone–releasing hormone; **VIP** - vasoactive intestinal peptide; **PHM/I** - peptide histidine methionine/isoleucine

GLUCAGON-FAMILY SEQUENCES	
GCG	HSQGTFTSDY SKYLDSRRAQ DFVQWLMNT
GLP-1	HAEGTFTSDV SSYLEGQAAK EFIAWLVKGR-NH_2
GLP-2	HADGSFSDEM NTILDNLAAR DFINWLIQTK ITD
GIP	YAEGTFISDY SIAMDKIHQQ DFVNWLLAQK GKKNDWKHNI TQ

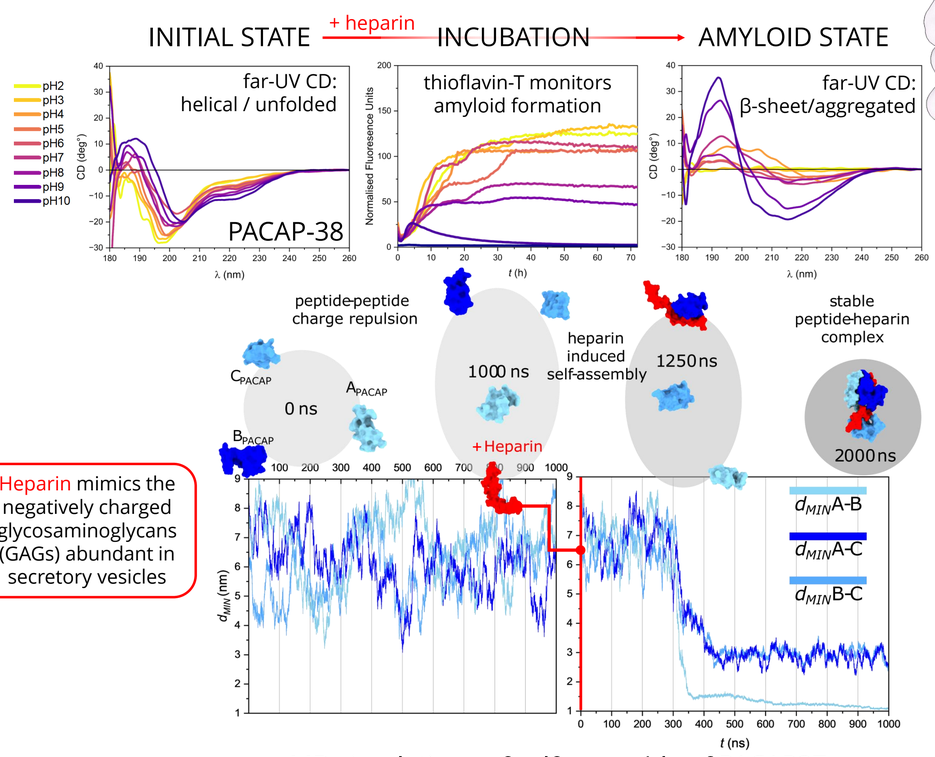
PACAP-FAMILY SEQUENCES	
PACAP-38	HSDGIFTDSY SRYRKQMAVK KYLAAVLGKR YKQRVKNK-NH_2
VIP	HSDAVFTDNY TRLRKQMAVK KYLNSILN-NH_2
PRP	DVAHGILNEAY RKVLDQLSAG KHLQSLVARG VGGSLGGGAGDDAEPLS
PHI	HADGVFTSDF SKLLGQLSAK KYLESLI
SCT	HSDGTFTSEL SRLREGARLQ RLLQGLV-NH_2
GHRH	YADAIFTNSY RKVLGQLSAR KLLQDIMSRQ QGESNQERGARARL-NH_2



WHAT DID WE FIND?

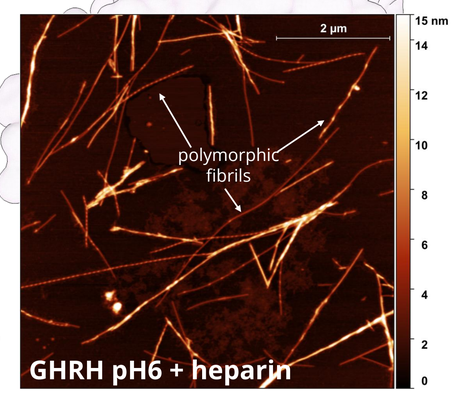
1. Glucagon-family peptides aggregate independently of heparin, whereas PACAP-family peptides require heparin for aggregation.

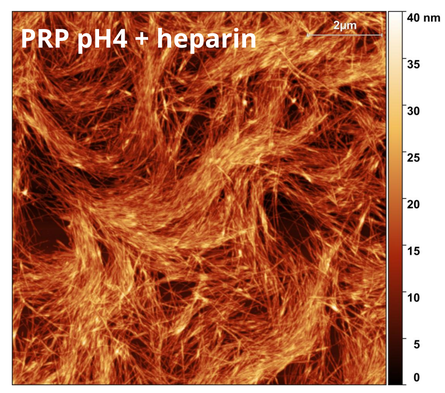
- 9/10 human hormones formed nanofibrillar amyloids at pH 4–6, close to secretory-granule conditions; secretin was the exception.
- Aggregation extended beyond this pH range, being generally favored at lower pH (pH < 5) but dependent on peptide charge distribution at higher pH (pH > 5).
- PACAP-family peptides required heparin for amyloid formation.
- MD simulations suggest that heparin recruits peptide monomers through electrostatic interactions, increasing their local concentration and promoting seed formation.
- Amyloid polymorphism occurred even within individual samples.



Heparin mimics the negatively charged glycosaminoglycans (GAGs) abundant in secretory vesicles

MD simulations of self-assembly of PACAP27



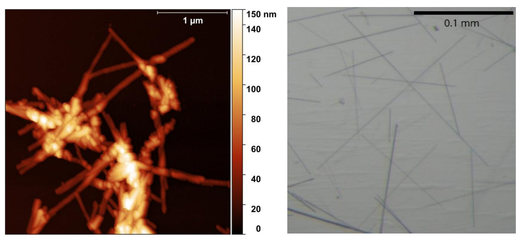


Atomic force microscopy micrographs of the amyloid fibrils

2. Amyloid formation is driven by sequence regions that also mediate receptor binding and activation.

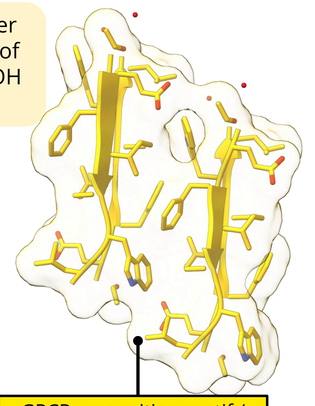
- Aggregation-prone hexapeptide regions (APRs) at conserved positions drive self-assembly and overlap with sequences involved in GPCR **binding** and **activation**.
- These hexapeptide segments form β-sheet-rich nano- and microcrystals with amyloid-like architecture revealed by X-ray crystallography.
- Aggregation propensity varies substantially between APRs:

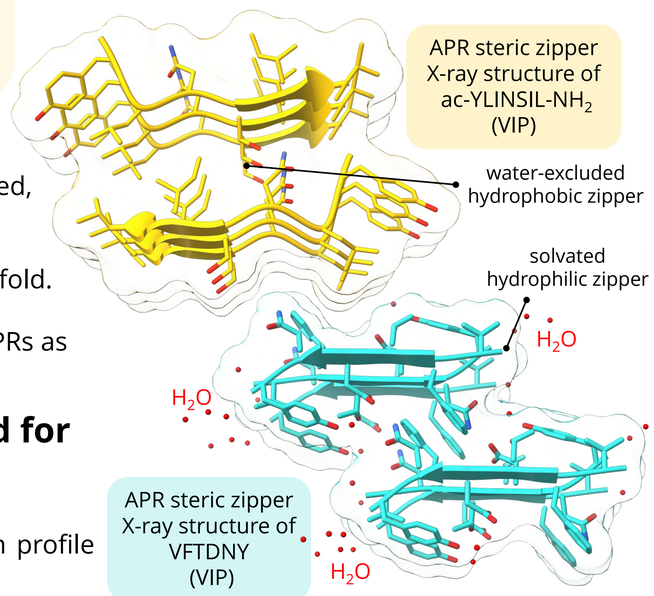
N-proximal APRs	**C-proximal APRs**
More polar	More hydrophobic
Similar sequence features across both families	Distinct sequence patterns between the two families
Weak aggregation propensity	Stronger aggregation propensity
Poorly predictedable	Well predictedable by aggregation predictors
Aggregate with protected APR termini	Glucagon family aggregate with free termini

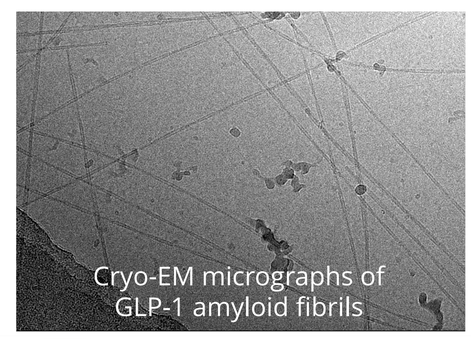


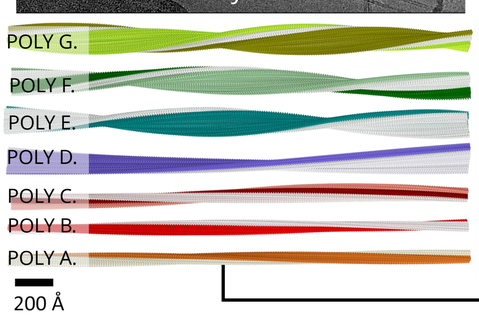
Nano- and microcrystals of hexapeptide ac-YLINSIL-NH_2 (VIP)

PDB-deposited APRs: 8ANJ; 8ANK; 8ONQ; 8ANN; 8ANL; 8ANH; 8ANM; 8ANI; 8ANG; 29VU; 29WR; 29VT; 29VS; 29VR; 29VQ; 29VP; 29VO

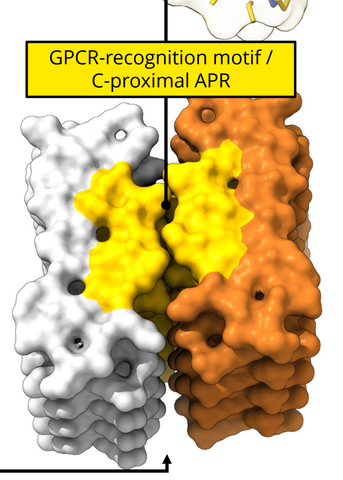








GLP-1 amyloid polymorphs



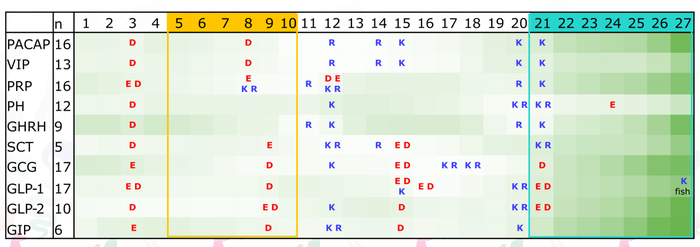
Cryo-EM structure of GLP-1 polymorph A (PDB deposited, not yet released)

- Aggregation is favored when amyloid gatekeeper Asp/Glu side chains are protonated, removing their negative charge.
- Tight steric zippers formed by diverse side-chain interactions stabilize the amyloid fold.
- Cryo-EM reveals pronounced GLP-1 polymorphism at two pH values, supporting APRs as key drivers of self-assembly.

3. The aggregation pattern of the family has been conserved for ~600 million years.

- Despite extensive evolution of receptor–ligand interactions (R3), the aggregation profile remained remarkably conserved across vertebrate evolution.
- PACAP–glucagon-related peptides from the extant protochordate *Chelyosoma productum* (R4), which diverged from the vertebrate lineage ~600 million years ago, retained this aggregation property.
- Early evolutionary divergence gave rise to distinct PACAP and glucagon families with chemically different amyloid gatekeeper patterns adjacent to the APRs.

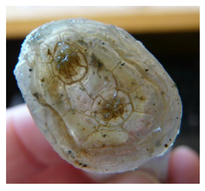
Gatekeepers in PACAP family	**Gatekeepers in glucagon family**
Lys/Arg dominance	Glu/Asp dominance
Positive charges limit self-assembly	Negative charges limit self-assembly
Less sensitive to pH changes	Protonation removes charge: →pH-sensitive aggregation
Heparin is required to neutralize these charges	Less sensitive to heparin



0 — Aggregation propensity — 1

Mean position-specific aggregation propensity across ***n*** species. Coloured residues indicate amyloid gatekeepers.

THE TAKE-HOME MESSAGE



Chelyosoma productum

- Our structural and genomic analyses reveal that amyloid formation is a more deeply conserved property of the PACAP–glucagon family than receptor specificity.
- Although this intrinsic aggregation propensity is often considered a “bug” in peptide synthesis and formulation, its remarkable evolutionary conservation suggests that it may in fact be a conserved biological feature.
- Understanding this functional, reversible amyloid state may therefore inspire novel strategies for peptide drug formulation.

OUR PAPERS

R1 R2 R3 R4

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