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# Optimization of Anti-Vasoconstricting UT-II-Derived Biogels

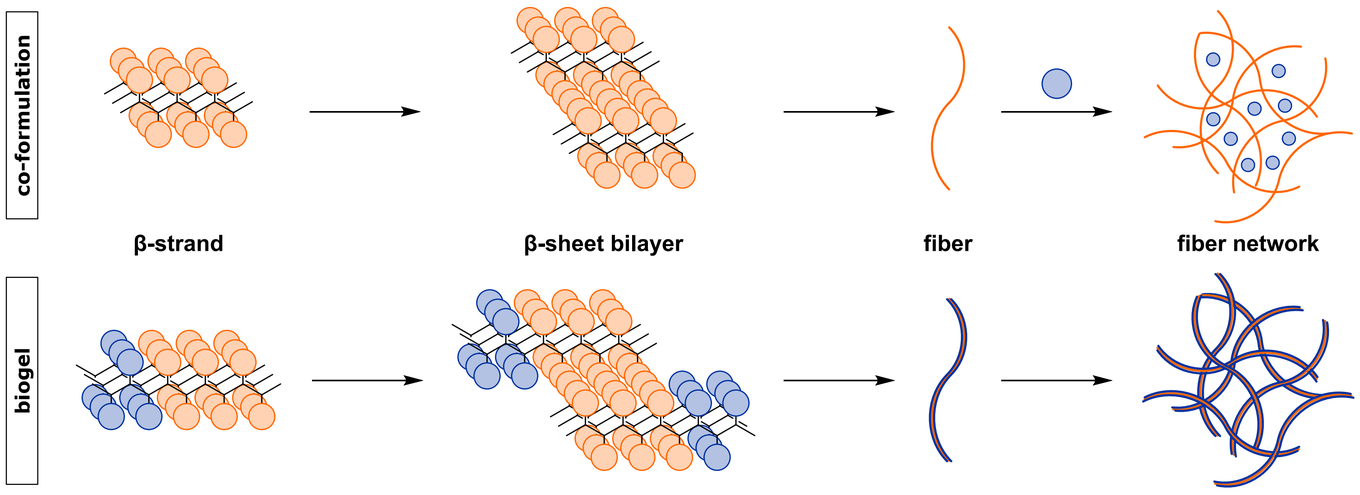
**Thibault Cauwenbergh[1], Juliana Dallagnol[2], David Chatenet[2], Charlotte Martin[1], Steven Ballet[1]**

*[1] Research Group of Organic Chemistry (ORGC) – Vrije Universiteit Brussel, Pleinlaan 2, Elsene (BE)*

*[2] INRS – Institut Armand-Frappier – Université du Québec, Québec (CA)*

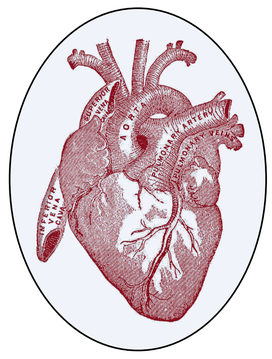
*e-mail: thibault.cauwenbergh@vub.be*

## CO-FORMULATION VS. BIOGEL FORMULATION [1]




Templated on our in-house developed amphipathic hexapeptide hydrogel (H-Phe-Gln-Phe-Gln-Phe-Lys-$NH_2$), we designed a **self-assembling hydrogel-drug conjugate** platform, termed *biogel*, with a proof-of-concept for the sustained release of opioid peptides. This **prodrug** approach offers an elegant solution to the often difficult-to-control and fast release properties characteristic for traditional co-formulation-based hydrogel platforms. The work presented here focuses on the implementation of the developed biogel concept in **targeting the urotensin-II receptor** (UT-IIR).

## UROTENSIN II RECEPTOR [2,3]



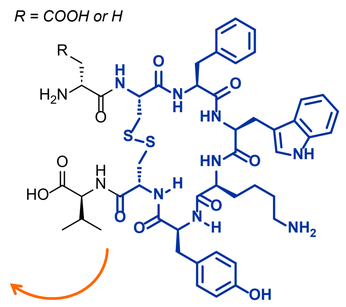
> G protein-coupled receptor
> somatostatin receptor homologue
> expressed in heart & pancreas
> intracellular $Ca^{2+}$ mobilization
> strong vasoconstricting effect
> involved in renal failure & diabetes

**hUT-II$_{(4-11)}$ & URP**

*Native agonists*
*Ultrapotent vasoconstrictors*

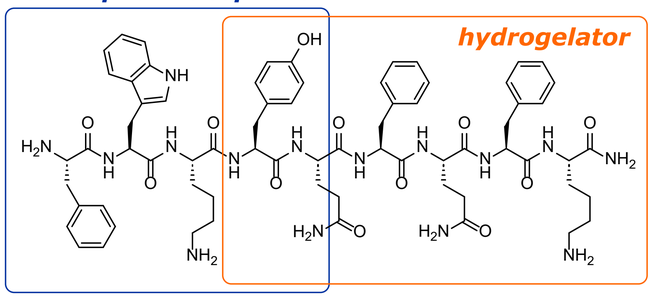
***Key ligand features***

- ✓ -FWKY- (or closely related) core motif
- ✓ -SS- cyclic hexapeptide
- ✓ Type II′ β-turn
- ✓ *N*- and *C*-terminal capping is tolerated




## LEAD BIOGEL DESIGN [4]

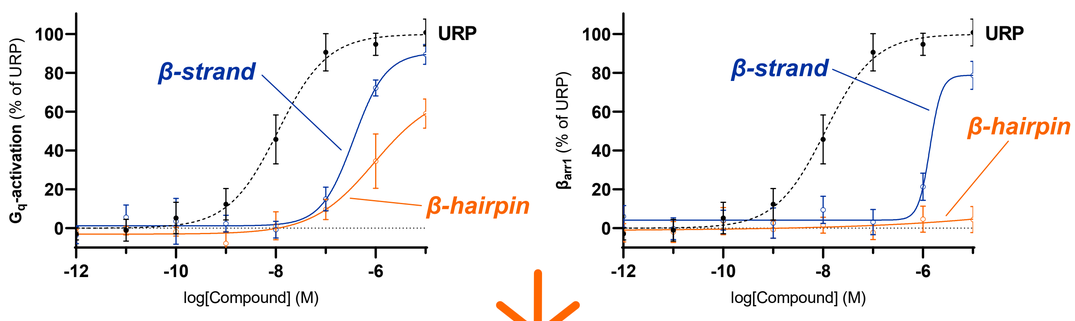
***UT-II pharmacophore*** — ***hydrogelator***

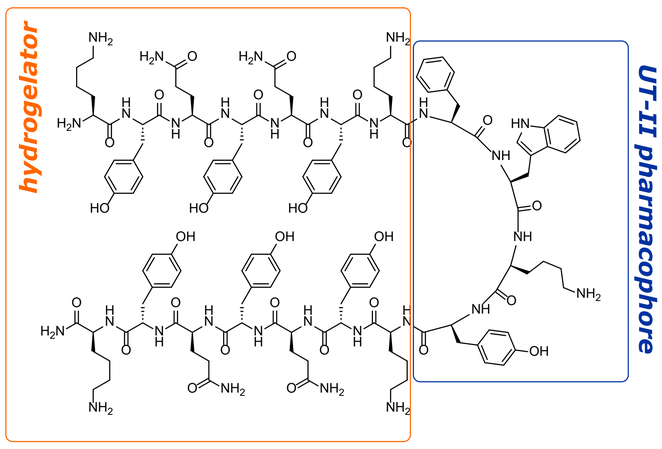


✓ **β-strand lead peptide**
*gel formation @ 2% w/v in PBS*

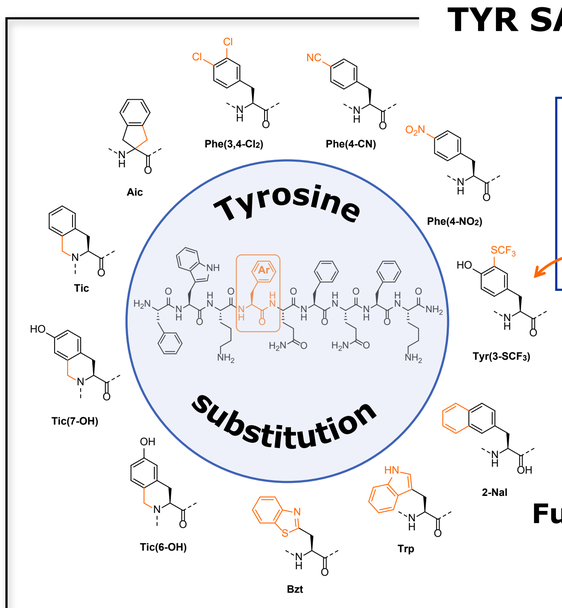
***β-STRAND*** ← ***GPCR activation*** → ***β-HAIRPIN***

(BRET biosensor assay on HEK293 cells)




## TYR SAR STUDY [5]




**General considerations**

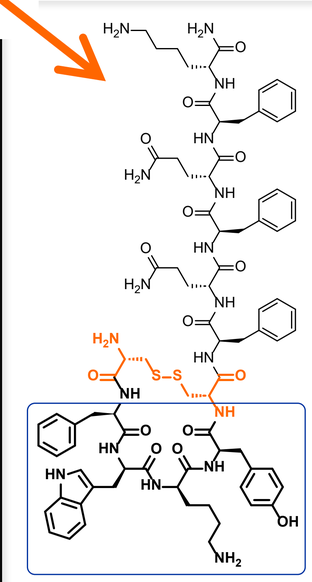
- ❑ **Phe** most tolerant residue
- ❑ **Trp** tolerant to stereochemical inversion
- ❑ **Lys** 3/4 carbon (primary) aliphatic amine!
- ❑ **Tyr** **aromatic moiety!**

Strong gel formation @ 2% w/v in PBS
**except** for constrained AAs (Tic(x-OH), Aic)

*however*

**Full loss of $G_q$ & $\beta_{arr1}$ activation observed...**
> *under investigation*

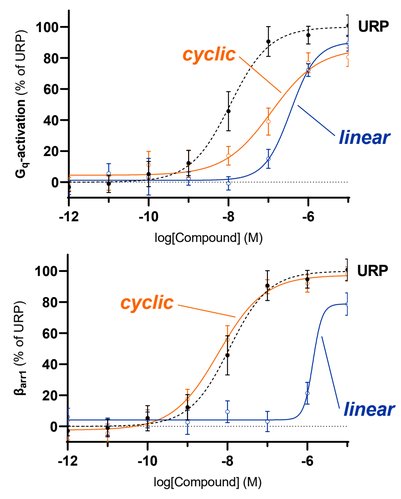
## CYCLIZATION [6]



***"Disulfide bridge is crucial for receptor recognition and activation"***

Slightly reduced gel strength
***but***
Improved activity!

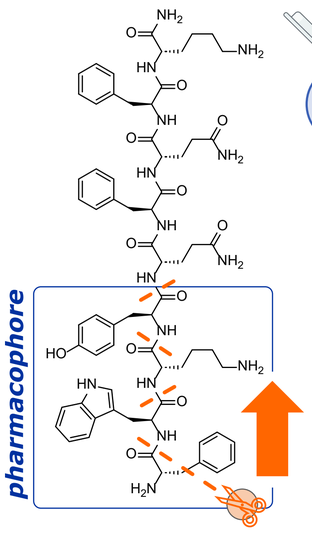
***GPCR activation***
(BRET biosensor assay on HEK293 cells)




***cyclization conditions***

- **on resin**
- $I_2$ (10 equiv.)
- **2% v/v anisole in NMP**
- **MW**
- **40 °C**
- **15 min**

## PROTEOLYTIC RESISTANCE



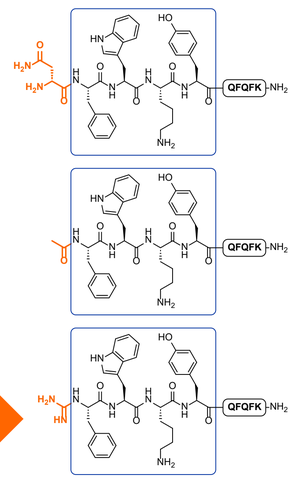

H-**FWKY**-QFQFK-$NH_2$
***fast degradation***
($t_{1/2}$ = 5.65 ± 0.26 min)

H-FQFQFK-**FWKY**-$NH_2$
($t_{1/2}$ < 2 min)

H-kfqfqf-**ykwf**-$NH_2$
($t_{1/2}$ = ?)

**NEED FOR MORE PROFOUND MODIFICATIONS**

### *N*-capping

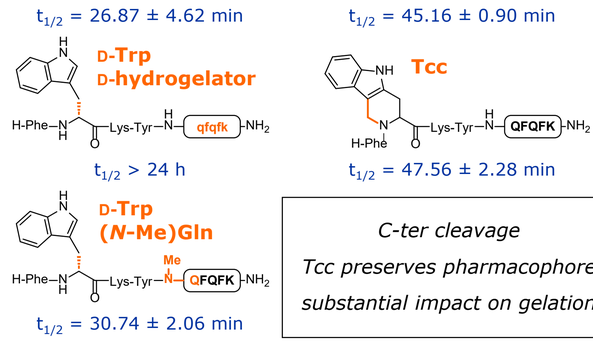



**D-Asn-**
$t_{1/2}$ = 93.59 ± 2.67 min
*C-ter cleavage*
*H-nFW-OH metabolite*

**Ac-**
$t_{1/2}$ = 70.64 ± 2.59 min
*C-ter cleavage*
*Ac-FWK-OH metabolite*

**Gdn-**
$t_{1/2}$ = 57.22 ± 3.80 min
*C-ter cleavage*
*Gdn-FWK-OH metabolite*

### Trp modification




**D-Trp**
$t_{1/2}$ = 26.87 ± 4.62 min

***(N*-Me)Trp**
$t_{1/2}$ = 45.16 ± 0.90 min

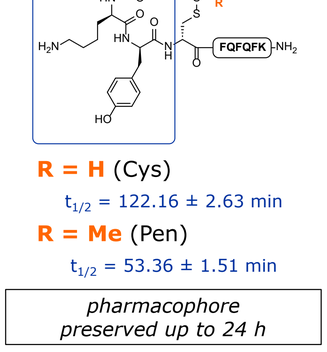
**D-Trp**
**D-hydrogelator**
$t_{1/2}$ > 24 h

**Tcc**
$t_{1/2}$ = 47.56 ± 2.28 min

**D-Trp**
***(N*-Me)Gln**
$t_{1/2}$ = 30.74 ± 2.06 min

*C-ter cleavage*
*Tcc preserves pharmacophore*
*substantial impact on gelation*

### Cyclization




**R = H** (Cys)
$t_{1/2}$ = 122.16 ± 2.63 min

**R = Me** (Pen)
$t_{1/2}$ = 53.36 ± 1.51 min

*pharmacophore preserved up to 24 h*

## CONCLUSIONS

> Search for highly active, long-acting UT-II-targeting conjugate hydrogel ('biogel')
> Cyclization of the pharmacophore enhances activity and proteolytic stability
> Unexpectedly, Tyr substitution yielded inactive linear peptide derivatives
> Proteolytic stabilization is needed to prolong peptide activity and preserve the pharmacophore

**PERSPECTIVES**

> Increase peptide activity, to compete with the parent peptide
> Test *in vitro* activity of the stabilized biogel analogues and their metabolites
> Further study & optimize the β-hairpin design
> Expand the biogel platform to other targets

## ACKNOWLEDGEMENTS

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## REFERENCES

[1] a) M. Bibian *et al.*, *J. Mater. Chem. B.* **2015**, *3*, 759–765; b) C. Martin *et al.*, *Med. Chem. Comm.* **2016**, *7*, 542–549; c) C. Martin *et al.*, *J. Med. Chem.* **2018**, *61*, 9784–9789.
[2] J. McDonald *et al.*, *J. Anesth.* **2007**, *21*, 378–389.
[3] a) R. Patacchini *et al.*, *Br. J. Pharmacol.* **2003**, *140*, 1155–1158; b) B. E. Maryanoff *et al.*, *J. Med. Chem.***2010**, *53*, 2695–2708.
[4] S. Bandholtz *et al.*, *J. Med. Chem.* **2016**, *59*, 10100–10112.
[5] a) D. Chatenet *et al.*, *J. Med. Chem.* **2006**, *49*, 7234–7238; b) b) A. Carotenuto *et al.*, *J. Med. Chem.* **2014**, *57*, 5965–5974; c) E. Billard *et al.*, *Biochem. Pharmacol.* **2017**, *144*, 100–107; d) J. Gregorc *et al.*, *J. Org. Chem.* **2023**, *88*, 13169–13177.
[6] a) A. Carotenuto *et al.*, *J. Med. Chem.* **2004**, *47*, 1652–1662; b) H. B. Zhang *et al.*, *Chin. Chem. Lett.* **2007**, *18*, 902–904.