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DESIGN OF GPR54 (KISS1R) CROSSLINKER PEPTIDES: NEW TOOLS TO STUDY G PROTEIN-COUPLED RECEPTORS (GPCR) LOCALIZATION AND FUNCTION

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Introduction. The neuropeptide kisspeptin and its receptor, GPR54 (KISS1R) play a central stimulatory role in mammalian reproduction. However, GPR54 localization is still incompletely mapped and its pharmacology only partially known. Receptor localization and receptor deorphanization remain important issues in drug discovery. To approach these problems, we designed new tools and used kisspeptin and its receptor to begin testing them.

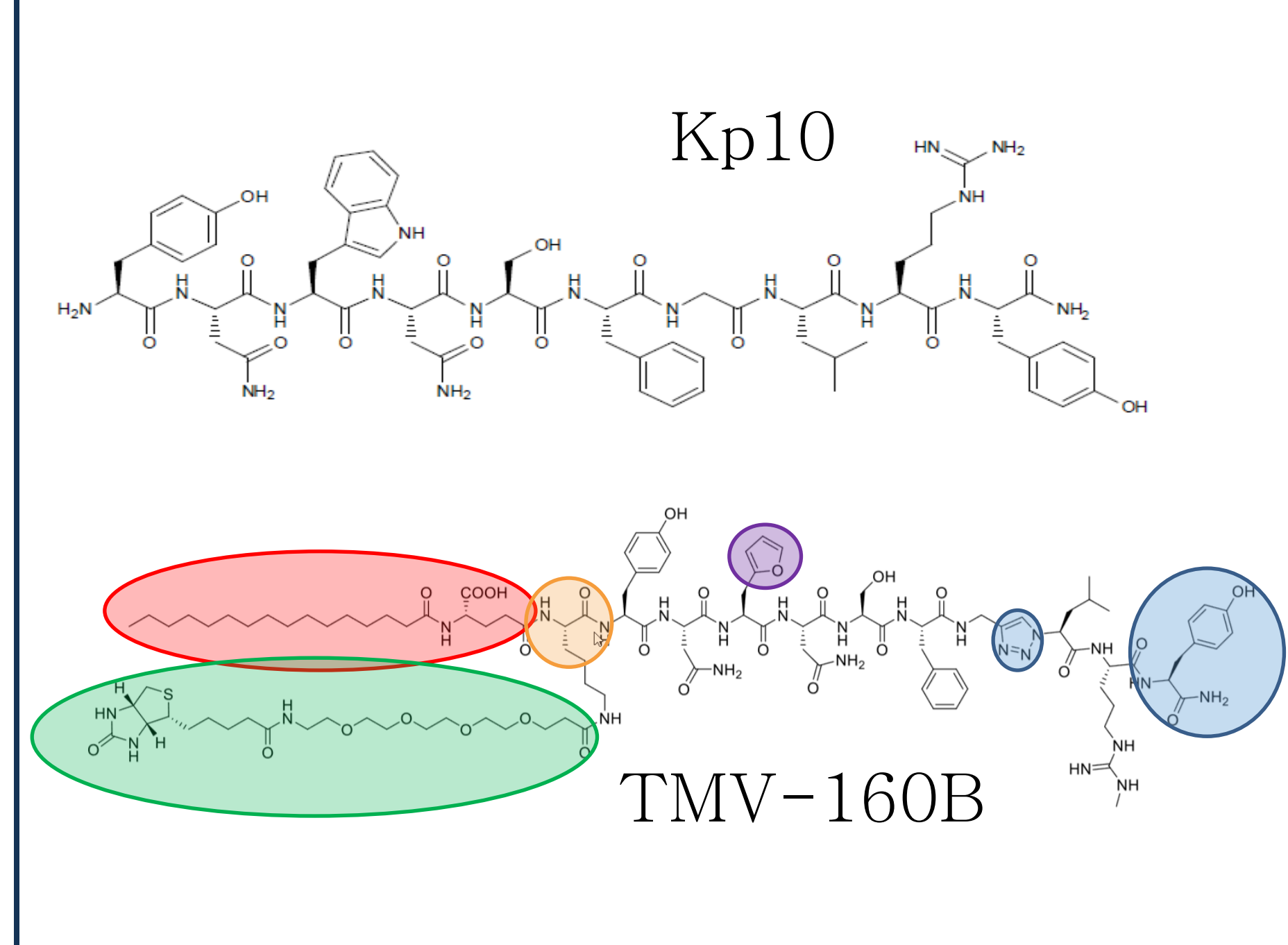
Materials and Methods. Four molecules (TMV-33-2-A, TMV-34, TMV-160A and TMV160B) were created based on the scaffold of the kisspeptin analog C6, that shows robust *in vivo* activity. These molecules bear modifications to improve the pharmacodynamics, to have tag (biotin insertion) and to bind covalently the receptor (furan insertion). *In vitro* activity of the new molecules was evaluated by a calcium mobilization assay in HEK-293 cells transfected with hGPR54. *In vivo* activity was assessed in adult C57BL6 male mice and in ovariectomized and estrogen replaced (implant) female mice. Molecules were injected ip with of test compounds (0,4 nmol/mouse) and

LH plasma concentration measured by an ELISA assay.

Sequence of human kisspeptin 10 and of modified peptides.

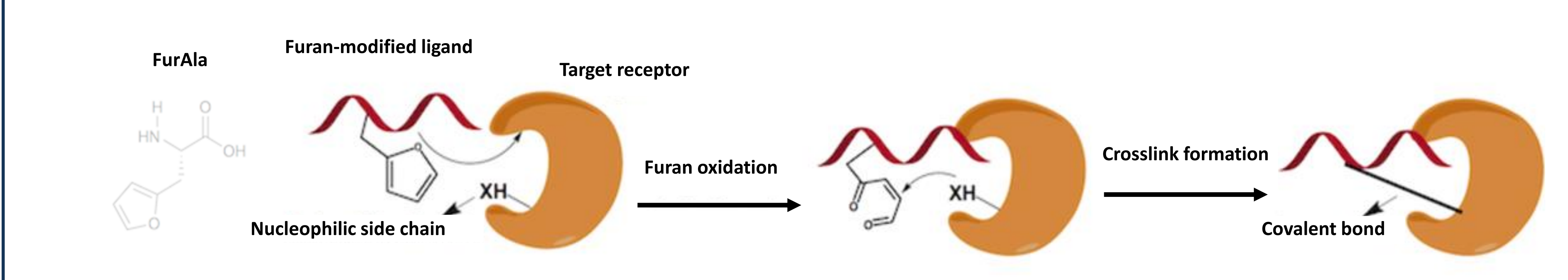
	H-Tyr-Asn- Trp -Asn-Ser-Phe- Gly -Leu-Arg- Phe-NH ₂	Kp10
	Palm-γ-Glu -Tyr-Asn- Trp -Asn-Ser-Phe- Glyψ[Tz] -Leu-Arg(Me)-Tyr-NH ₂	C6
	Palm-γ-Glu -Tyr-Asn- FurAla -Asn-Ser-Phe- Glyψ[Tz] -Leu-Arg(Me)-Tyr-NH ₂	TMV-33-2A
	Biotin-linker -Tyr-Asn- FurAla -Asn-Ser-Phe- Glyψ[Tz] -Leu-Arg(Me)-Tyr-NH ₂	TMV-34
Biotin-linker	Lys -Tyr-Asn- Trp -Asn-Ser-Phe- Glyψ[Tz] -Leu-Arg(Me)-Tyr-NH ₂	TMV-160A
Palm-γ-Glu		
Biotin-linker	Lys -Tyr-Asn- FurAla -Asn-Ser-Phe- Glyψ[Tz] -Leu-Arg(Me)-Tyr-NH ₂	TMV-160B
Palm-γ-Glu		

Structure of the human kisspeptin 10 and of modified peptides



The modifications introduced into the human kp10 are indicated in color in the amino acid sequence. These modifications allow: i) **protection against enzymatic degradation**, ii), **reduced renal excretion**, iii) **to tag the molecule** and iv) **to bind covalently the receptor**. Lysine is present **as a chemical moiety to anchor both** biotin and palmitoyl.

Scheme showing the structure of FurAla and the general principle of furan crosslinking

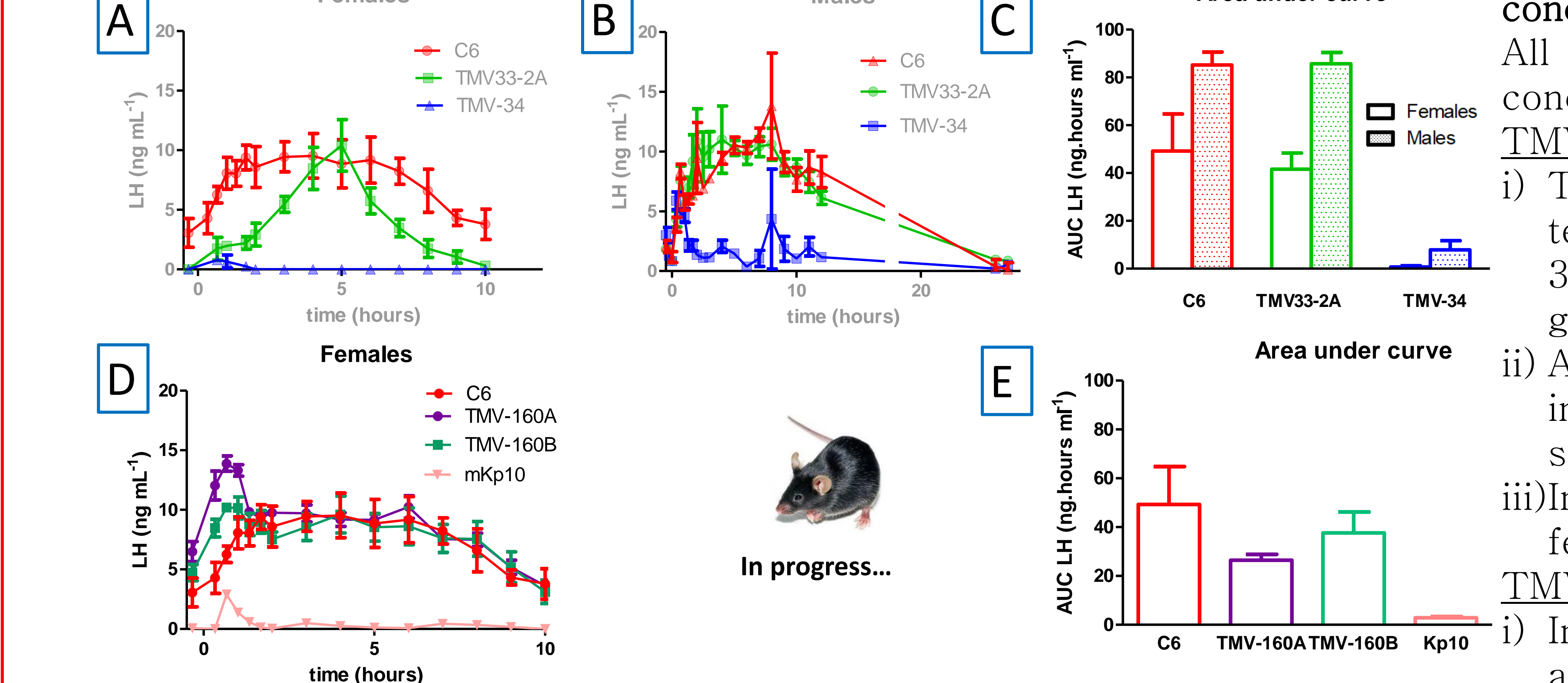


In vitro activity

Compound	EC ₅₀
hKp10	2,9.10 ⁻¹⁰
C6	2,08.10 ⁻¹⁰
TMV-33-2A	7.10 ⁻⁹
TMV-34	5.10 ⁻¹¹
TMV-160A	<1.10 ⁻¹²
TMV-160B	<1.10 ⁻¹²

The new analogs are potent receptor agonists.

In vivo model (mouse)



Effect of molecules on LH plasma concentration:

All molecules increased LH plasma concentration in both sexes. **TMV-33-2A and TMV-34**:
 i) TMV-34, bearing a biotin at the N-terminal, had a shorter effect than TMV-33-2A, harboring a palmytoyl-isoglutamyl at the N-terminal.
 ii) Amplitude and duration of LH increased induced by TMV-33-2a and C6 were similar for males (B).
 iii) In males total AUC is larger than in females for both molecules (C). **TMV-160A and TMV-160B**:
 i) In females the kinetic of the LH increase after TMV-160A and B is similar to that induced by C6. However, their AUC is marginally smaller.
 ii) Both molecules induced a secretion of LH broader than native Kp10.

Kinetic of LH concentration for different compounds in females (A, D); males (B, E). Area under curve calculated. However, their AUC is marginally smaller.

Conclusions. These preliminary results suggest that it is possible to develop highly potent and selective crosslinker molecules as new tools to study the kisspeptin system. More importantly, this strategy holds the promise to be applicable to study other targets. Structural optimization is in progress to evaluate various combinations of chemical modifications and different tags to allow *ex vivo* receptor localization by crosslinking the receptor with the tagged analog.

