A MULTIMODAL MOLECULAR TOOLBOX FOR IN VITRO AND IN VIVO STUDIES OF THE OXYTOCIN RECEPTOR



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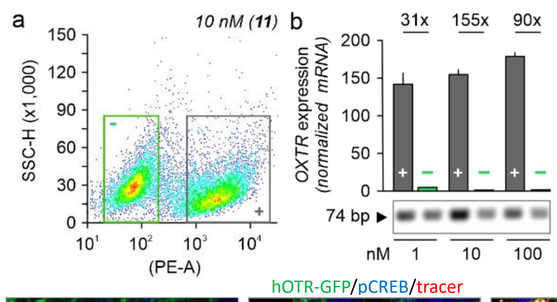
Significance & Aims

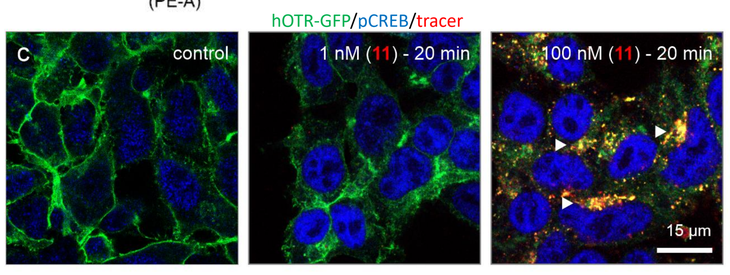
The oxytocin receptor (OTR) is a G protein-coupled receptor (GPCR) central to reproduction and social behavior and a promising target for studying neuropsychiatric disorders and cancer.[1] However, progress has been limited by an incomplete understanding of OTR expression and function across tissues, creating a need for tools that can probe the receptor at multiple biological scales.[2,3] To address this gap, we are developing a **multimodal molecular imaging platform** based on peptide-derived tracers for both fluorescence and positron emission tomography (PET) applications. Together, **these tools aim to provide a comprehensive framework for characterizing OTR biology and accelerating the development of next-generation OTR-targeted theranostics**.

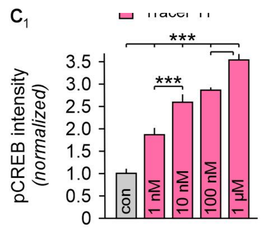
Fluorescent Tracers

Fluorescent agonist tracers were tested in various experimental set-ups,[4] such as **fluorescence-activated cell sorting (FACS) and *in vitro* OTR detection and activation**.

Tracer application to a mix of hOTR- and $hV_{1a}R$-expressing HEK293 cells resulted in successful sorting as confirmed by RT-qPCR (***a,b***). Incubation of HEK293 cells expressing hOTR-GFP with tracer resulted in receptor internalization (*arrowheads*) and dose-dependent phosphorylation of cAMP-responsive element binding protein (pCREB) (***c-c₁***).

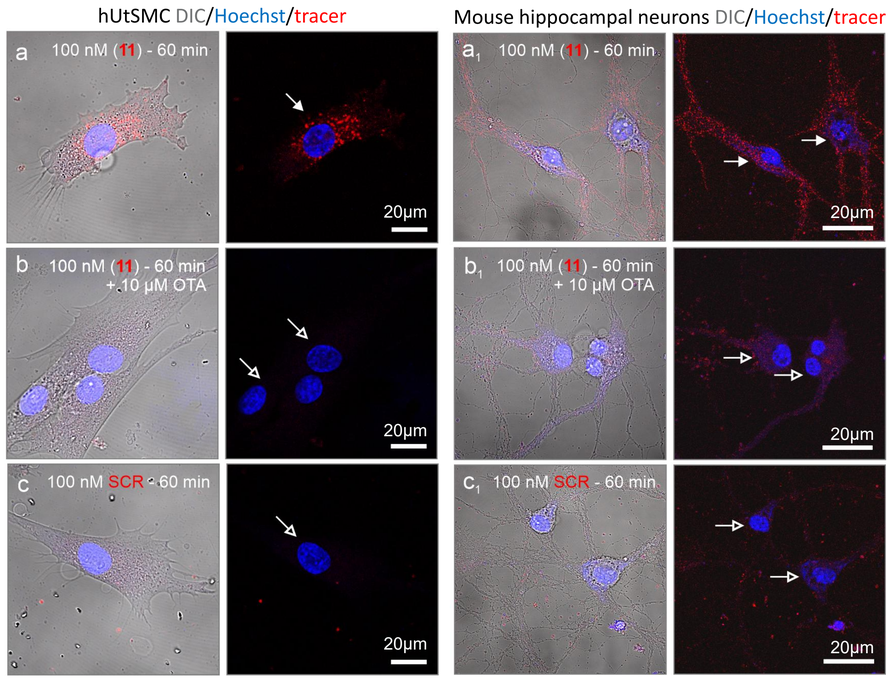






hUtSMC DIC/Hoechst/tracer

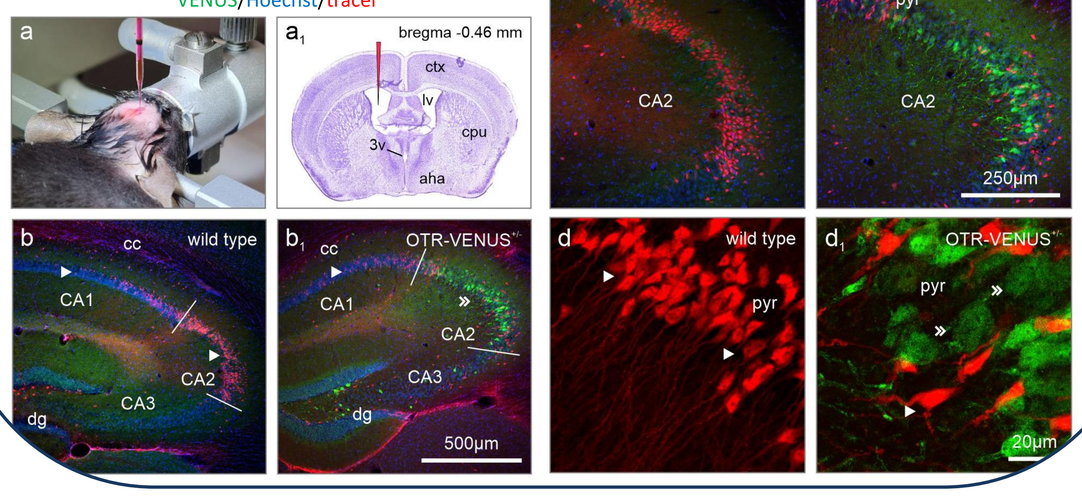
Mouse hippocampal neurons DIC/Hoechst/tracer



In human uterine smooth muscle cells (hUtSMC) and mouse neurons, the tracer **labeled endogenous OTR expression sites** (*arrows* in ***a, a₁***). Labeling was blocked by peptide antagonist OTA, and a tracer with **scrambled amino acid sequence SCR** did not label OTR (*empty arrows* in ***b-c₁***).

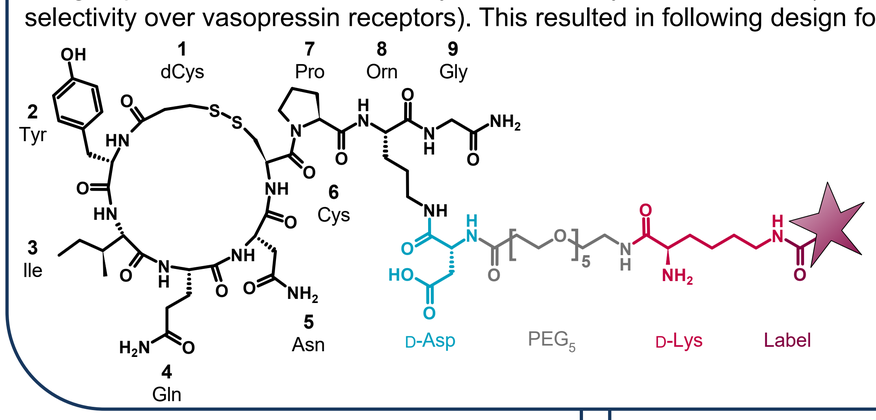
Intracerebroventricular injection of tracer into the mouse brain resulted in labeling of OTR-expressing neurons (***a,a₁***). After 2 hours, tracer was detectable in the CA1, CA2, and dg of the hippocampus (*arrowheads*, ***b,c,d***). In the OTR hypomutant, tracer signal was reduced significantly, indicating OTR specificity (*double arrows*, ***b₁,c₁,d₁***).

VENUS/Hoechst/tracer



Approach

Tracers consisting of **peptide backbone, functional linker, and labeling group** were prepared *via* manual Fmoc-solid phase peptide synthesis (Fmoc-SPPS) and solution phase labeling group attachment. Compound pharmacology was determined in radioligand binding assays and the design optimized until desired affinity and selectivity were achieved (~1 nM OTR affinity, >100-fold selectivity over vasopressin receptors). This resulted in following design for fluorescent tracers:



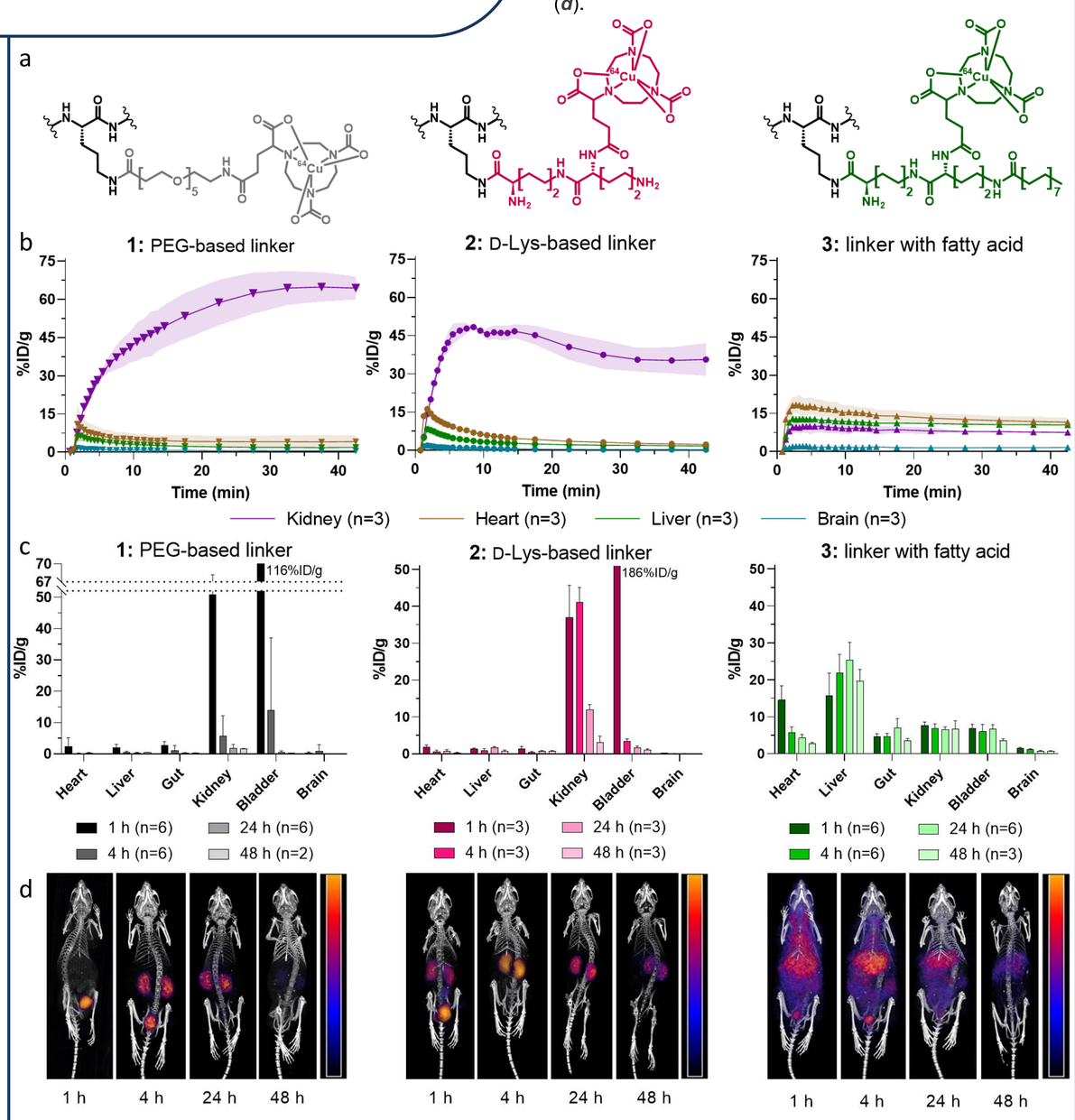
Patented functional linker fulfils multiple tasks *via* modular design:

i) 100-fold selectivity
ii) Distance from binding pocket
iii) Tracer fixability
iv) Handle for labeling group attachment

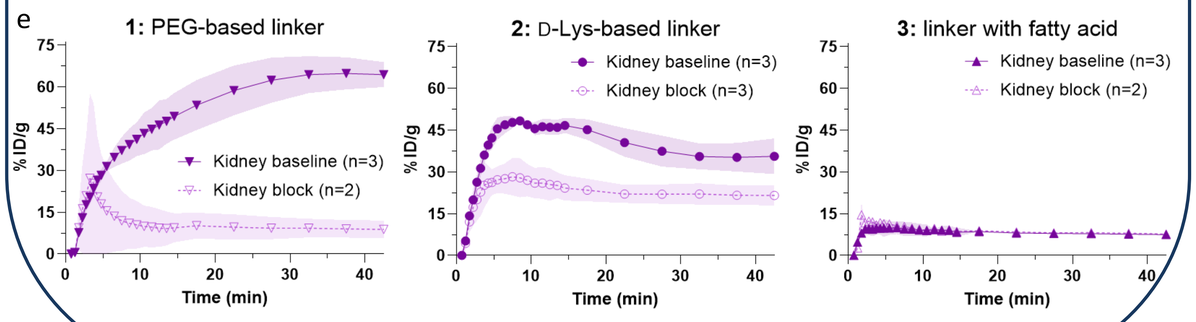
PET Tracers

Compared to fluorescent tracers, PET tracers additionally require the optimization of **pharmacokinetic properties** (proteolytic stability, clearance rates, tissue distribution and retention, etc). We are currently in the process of optimizing PET tracer design by testing the impact of different linker components on tracer performance in healthy mice *in vivo*.

Three PET tracers with different linkers (***a***) were labeled with copper-64, injected into the tail vein of healthy mice, and their distribution followed on a PET/CT scanner (***b-d***). **Clear differences in biodistribution are observable depending on the type of linker** both within the first hour of injection (***b***) as well as after up to 48 h (***c***), with lipidated tracers having prolonged circulation half-lives. Representative PET/CT images of mice for are depicted in (***d***).



Additionally, the impact of **receptor blocking** by preinjection of cold OT (2 mg/kg) 30 min prior tracer injection was tested with signal reduction across all observed tissues. Most significant differences were obtained for the kidneys (***e***), likely due to saturation of kidney-expressed transporters.



Conclusions & Outlook

Our selective fluorescent tracers enable high-resolution OTR detection through confocal microscopy and flow cytometry and support real-time visualization of receptor dynamics using live-cell single-molecule imaging. They are highly useful for **receptor localization, activation, and trafficking** studies as well as **FACS**.[4] Future experiments encompass mapping of OTR at high resolution in different tissues as well as the design of antagonistic and hyperfluorescent tracers. For non-invasive imaging, PET tracers are being developed to **study OTR expression *in vivo*.** Initial biodistribution studies were performed in healthy mice, and ongoing efforts focus on optimizing linker design to enhance pharmacokinetics, target tissue uptake, and specificity. PET tracers will also be applied in cancer models. Overall, our multidisciplinary approach holds promise of shedding light on the intricate role of OTR in various diseases, potentially paving the way for new therapies.

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