# oGPCR decision tree

Failed modeling or no actives

enabled GPCR?

High seq id to xtal-

Standard oGPCR (~85%)?

determine signaling, physically screen for lead matter

Build homology models to enrich lead matter from decoys

Large library docking, test ~40



actives?



Optimize oGPCR probe, with favorable PK, determine activity against mouse oGPCR; if favorable activity consider mouse testing

TASR, VNR, ADGRG, FZD families

- CRISPR/CLARITY to illuminate distribution and potential function
- Identify signaling via CRISPR-cells
- Consider
   DREADD
   depending on IRG working
   group

# Flowchart: Med chem optimization oGPCRs

~40 obtained Validated by MS Tested in functional assays



Actives used to refine models and docking

Mutagenesis to verify docking pose

#### Progress:

- Potency 1 µM or better
- GPCR-ome profiling limited off-targets
- PDSP profiling limited off targets
- Preliminary SAR validated by re-synthesis

## 10-20 analogues to explore SAR

Analogues verified



# Triage:

• SAR/potency plateau

No actives

Synthesis challenging

# To Sigma Catalogue

- Active and inactive probe pair 100 nM potency or better
- Minimal off-target GPCR-ome activity
- Favorable PDSP off-target profile

**UCSF** 

UNC

Mt. Sinai

**PDSP** 

#### Options:

- CRISPR/CLARITY/DREADD
- Re-do physical and computational screens
- De-prioritize target

In vivo testing

- Favorable PK
- KO available
- Consider outside collaborators

# CRISPR-Clarity-DREADD

**UNC** (Roth lab, Genetics and Pathology Cores

**UCSF** and KMC

**IDG Working Group** 

TASR, VNR, ADGRG, FZD families and oGPCRs failed modeling

Targets prioritized by IDG Working Group

#### CRISPR knock-in design:

- Epitope tag (FLAG, HA) to minimize expression
- **IRES-Cre**

- Initial knock-in fails:
  Redesign targeting guide RNAs
  May iterate x 2 before de-prioritization of target

### Targeting achieved

- F1 obtained (UNC Genetics Core)
- Bred to reporter line (Roth lab)
- Survey of tissue distribution (Roth Lab + UNC pathology core)

Input from IDG-WG

- Which targets to prioritize for survey vs in-depth interrogation
- DREADD go/no-go Further anatomical and functional characterization:
- Whole body, single-cell resolution via iDISCO/CLARITY (UNC-Cal Tech collaborators)
- Deposition of images (UCSF/KMC)
- DREADD-based activation of signaling to elucidate physiology
- Videos of physiology deposited (UCSF/KMC)