Direct Observation of the Interaction of the Sigma-1 Receptor with ASIC1a

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INTRODUCTION

ASICs

- Proton-gated cation channels
- Several known (1a, 1b, 2a, 2b, 3 and 4)
- ASIC1a forms highly Ca^{2+}-permeable homotrimer
- Mediates neuronal damage post-stroke via toxic influx of Ca^{2+} ions

Sigma Receptors

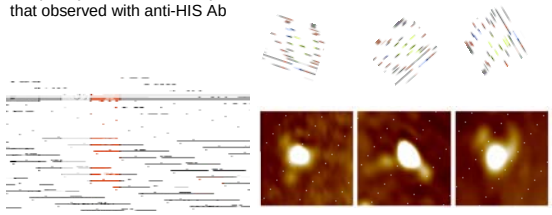
- No sequence homology with any known mammalian protein
- Implicated in a range of psychological conditions
- Modulates several neuronal ion channels
- Two known: σ1 and σ2
- σ1 down-regulates ASIC1a-mediated Ca^{2+} entry following stroke
- Mode of ASIC1a-σ1 interaction previously unknown

ASIC1a + Sigma1 Receptor

ASIC1a expressing cells were transiently transfected with σ1 Receptor bearing HIS_6 and FLAG tags

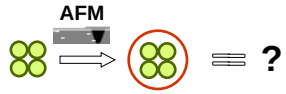
Angle distribution suggests each ASIC1a subunit is bound by one σ1 Receptor

Frequency of decoration very similar to that observed with anti-HIS Ab

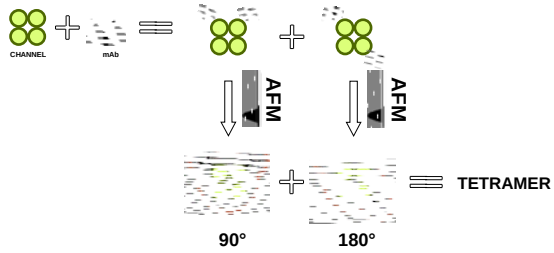


STOICHIOMETRY BY AFM

Resolution of AFM is insufficient to reliably resolve the subunits of multimeric ion channels, for example:

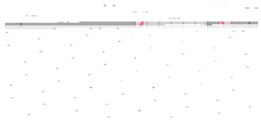


Decoration of epitope-tagged subunits with **antibodies** overcomes this limitation: the angles between 2 bound antibodies reveal stoichiometry:

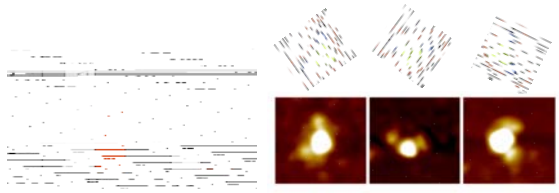


ASIC1a + Sigma1 Receptor

Direct binding of σ1 Receptor to ASIC1a confirmed by imaging of ASIC1a-σ1 Receptor complexes incubated with **anti-FLAG** Ab



Specificity confirmed by use of anti-V5 Ab (-ive control) where ~1/10th frequency of double decoration was observed

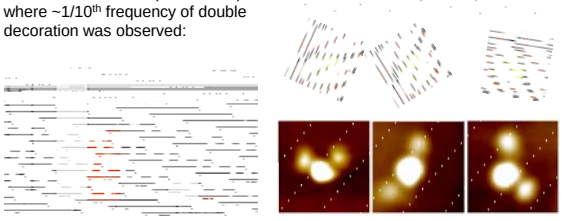


ASIC1a

HEK cell line was stably transfected with ASIC1a bearing a HIS_8-tag

Imaging of HIS_8-tagged ASIC1a incubated with **anti-HIS Ab** confirms trimeric structure:

Specificity of Ab binding confirmed by use of anti-FLAG Ab (-ive control) where ~1/10th frequency of double decoration was observed:



Conclusions

- σ1 Receptor interacts with ASIC1a through direct physical contact
- Stoichiometry of this interaction is 1 σ1 Receptor:1 ASIC1a subunit

Future work

- Study effects of σ1 Receptor ligands on ASIC1a binding
- Study influence of σ1 Receptor binding on ASIC1a plasma membrane localisation and lateral diffusibility

Acknowledgements

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