CHILDREN'S MEDICAL RESEARCH INSTITUTE

Jeans for Genes®

THE UNIVERSITY OF NEWCASTLE AUSTRALIA

Endocytosis Modulator Program

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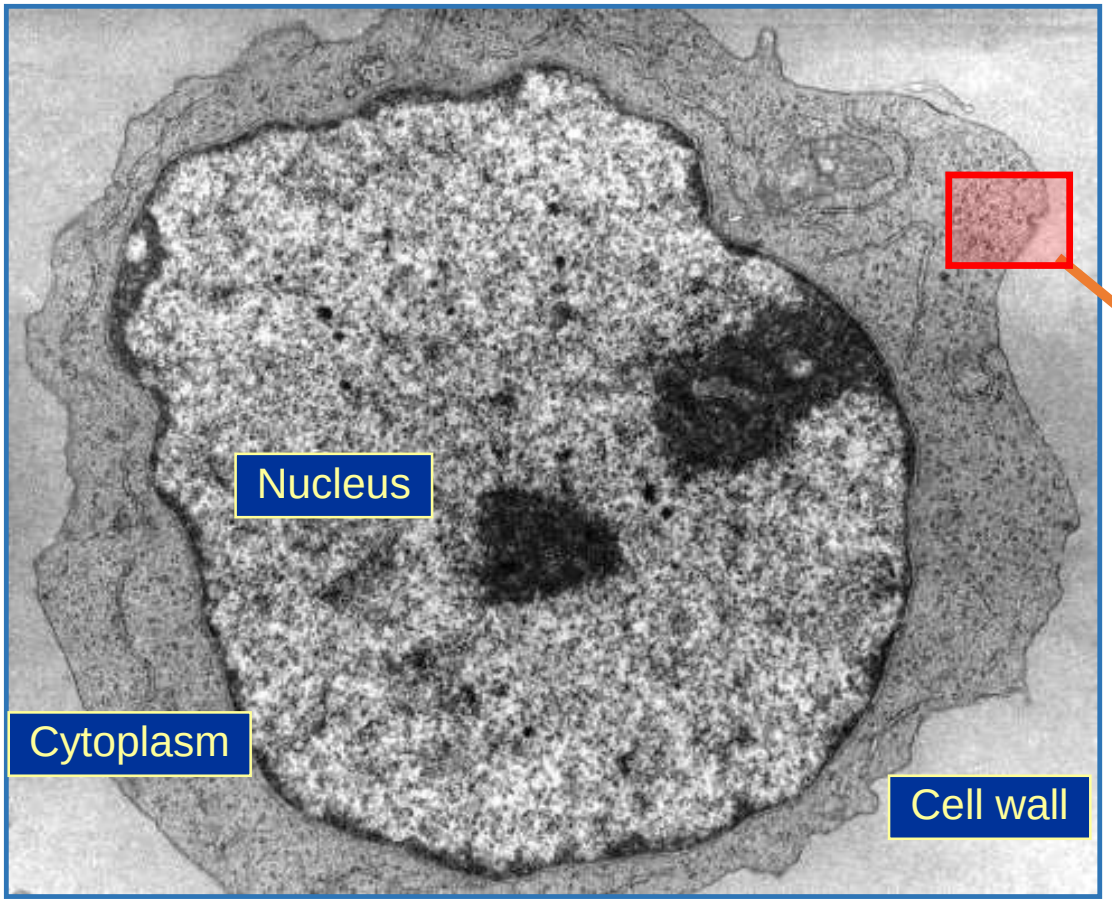
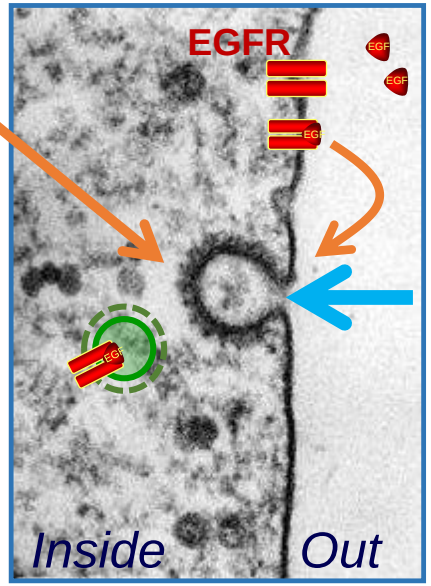
Dynamin Medicinal Chemistry

Endocytosis Controls Proteins On Cell Surface



Whole cell

EGFR is normally on the cell surface



EGFR can be internalized by endocytosis

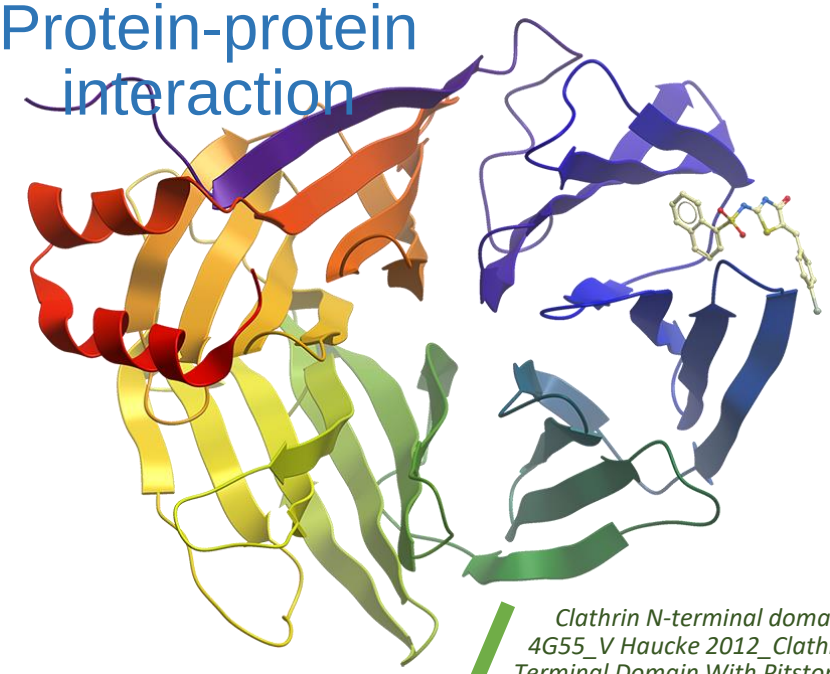
Endocytosis
of vesicles

Endocytosis regulates: receptor signalling, and what the immune system can ‘see’

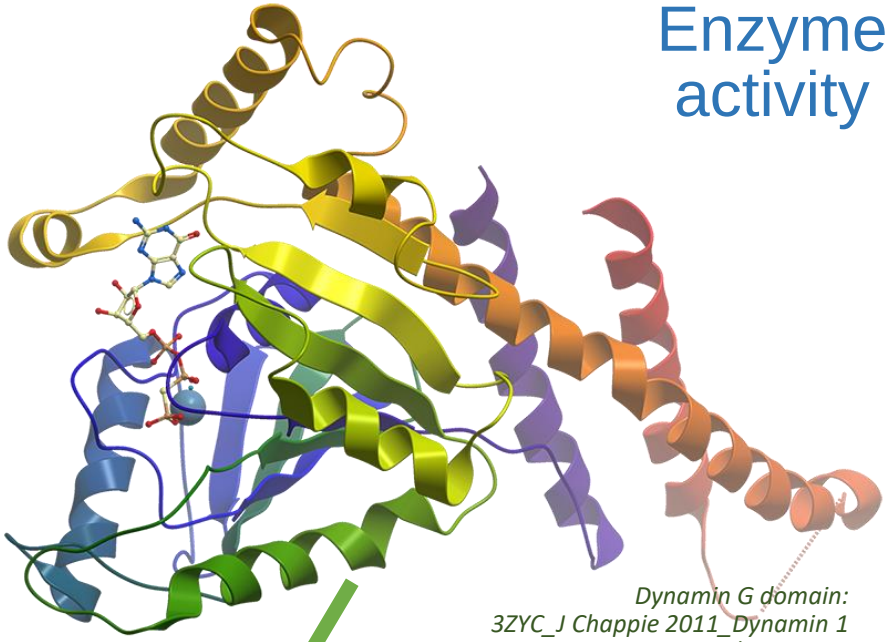
Clathrin and Dynamin

Protein-protein interaction

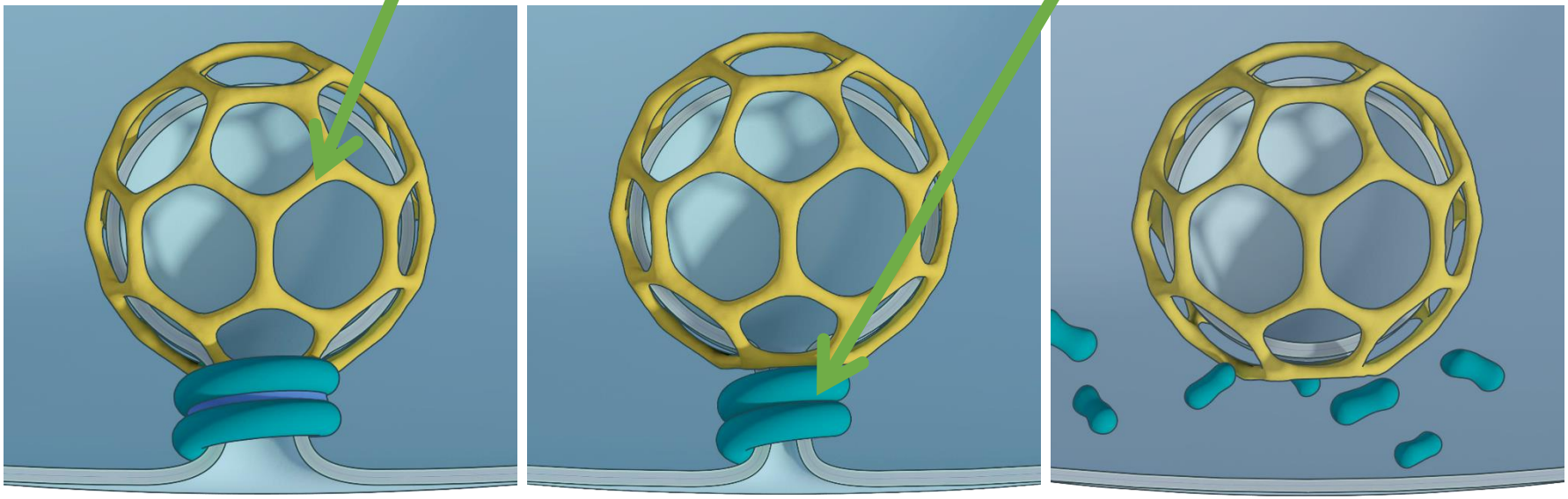
Enzyme activity



Clathrin N-terminal domain:
4G55_V Haucke 2012_Clathrin Terminal Domain With Pitstop 2



Dynamin G domain:
3ZYC_J Chappie 2011_Dynamin 1 GTPase GED Dimer With GMPPCP



Screening Platform For Dynamin Modulators

in vitro screens

dyn I	dyn II
dynI (rec human)	*dynII (rec human)*

\+

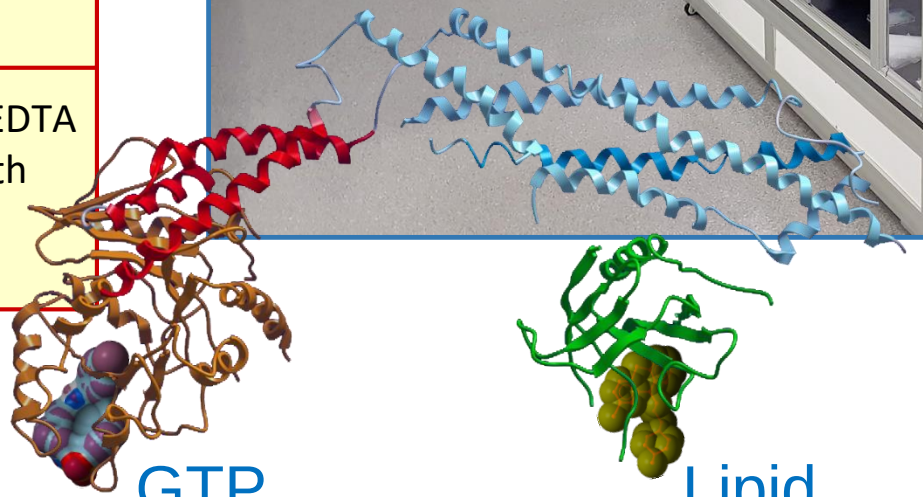
Lipids
(or MTs, or Grb2)

\+

Mg^{2+}-GTP

GTP → GDP + P_i (30 mins, 37°C)

- Stop reaction with EDTA
- Color developed with **Malachite Green**
- Absorbance 650 nm



GTP

Lipid

Ngoc Chau, Ainslie Whiting

CHILDREN'S MEDICAL RESEARCH INSTITUTE
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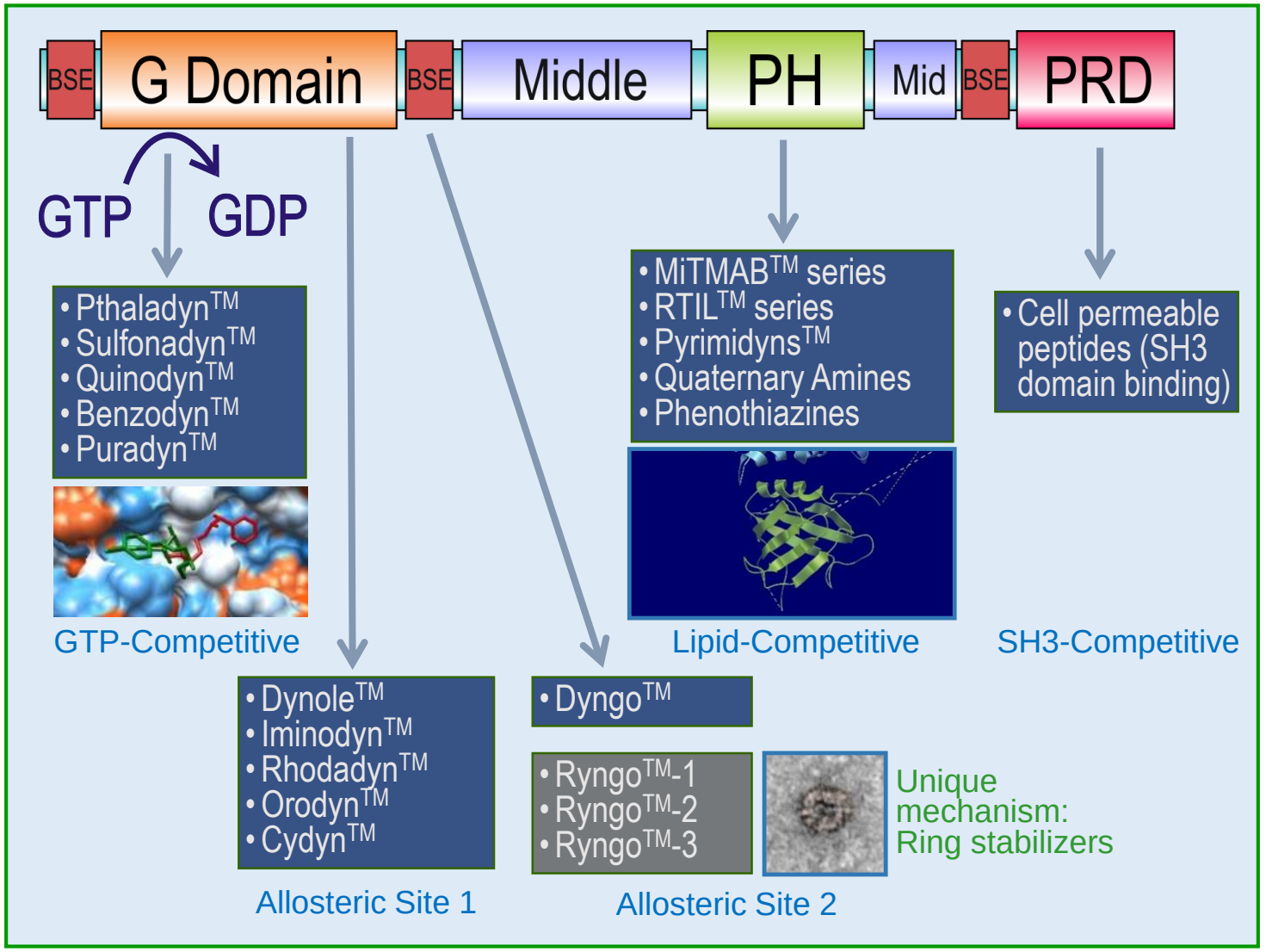
DRUG SCREENING FACILITY

Dynamin Modulators: Distinct Mechanisms

Our team has 47 joint publications on dynamin modulators



Robertson et al (2014) Nature Protocols 9, 851

Screening Platform For Dynamin Modulators

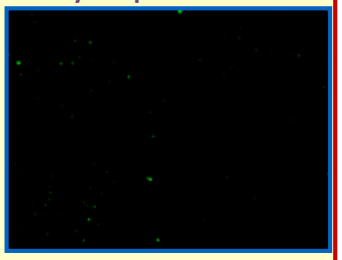
In-cell screens

dyn I

SVE in rat brain nerve terminals

Synaptosomes

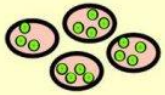
FM 4-64

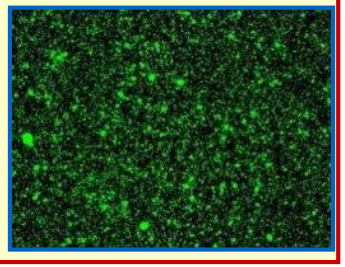


+/- drugs

KCl depol 2 min

ADVASEP-7 block





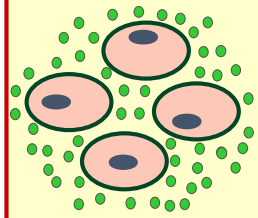
Synaptosomes: <1 µm

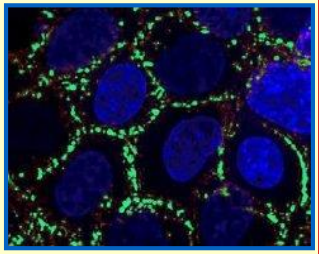
dyn II

CME in U2OS bone osteosarcoma

EGF-A488 or Tf-A594

U2OS cells

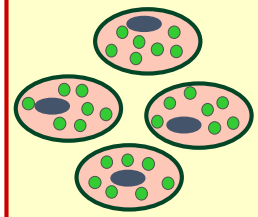


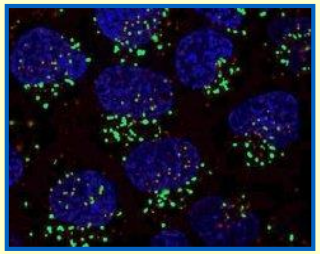


+/- drugs

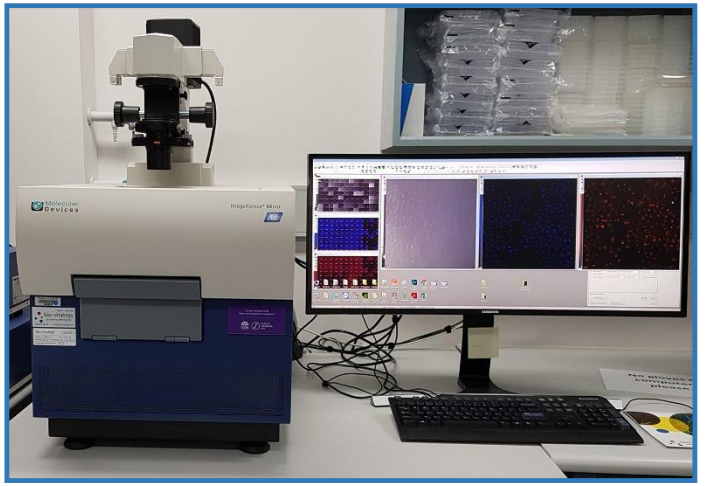
8 min incubation

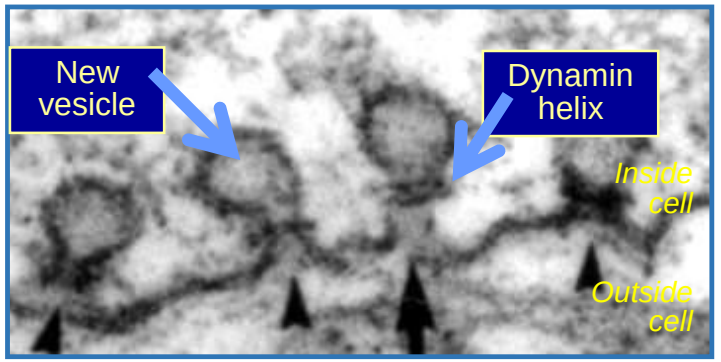
Acid wash



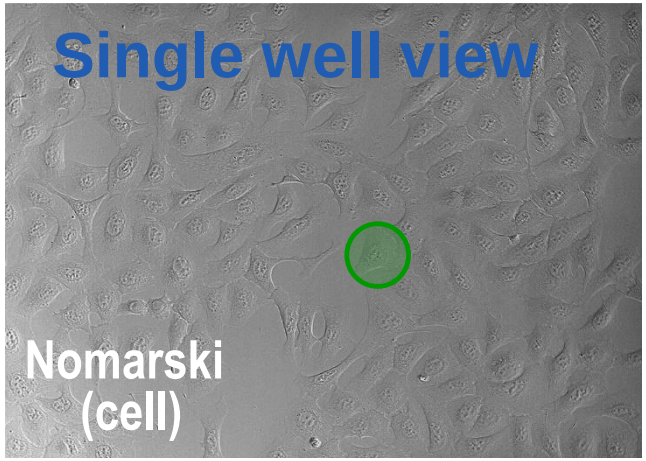


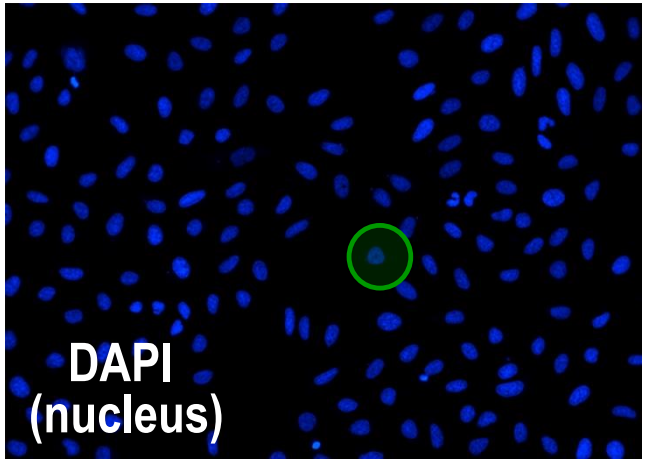
Cells: >20 µm

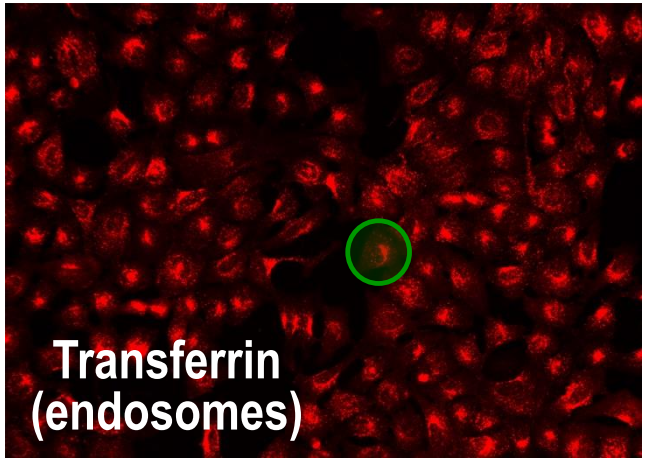




Functional Assay Example - Endocytosis

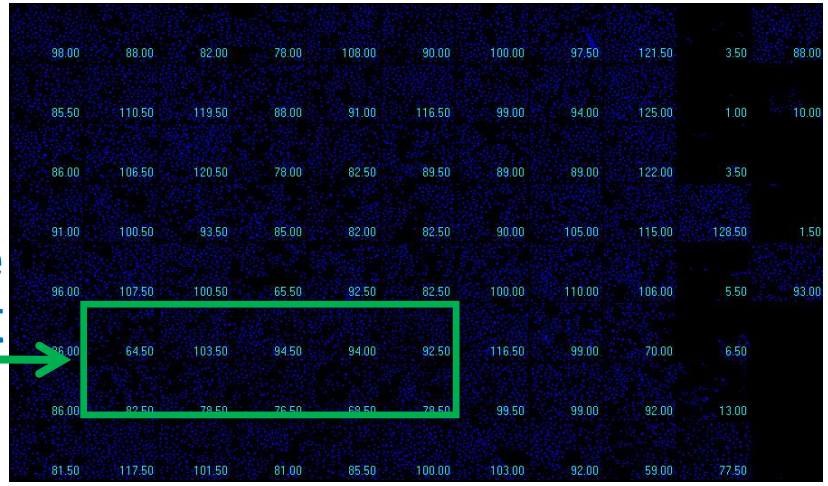




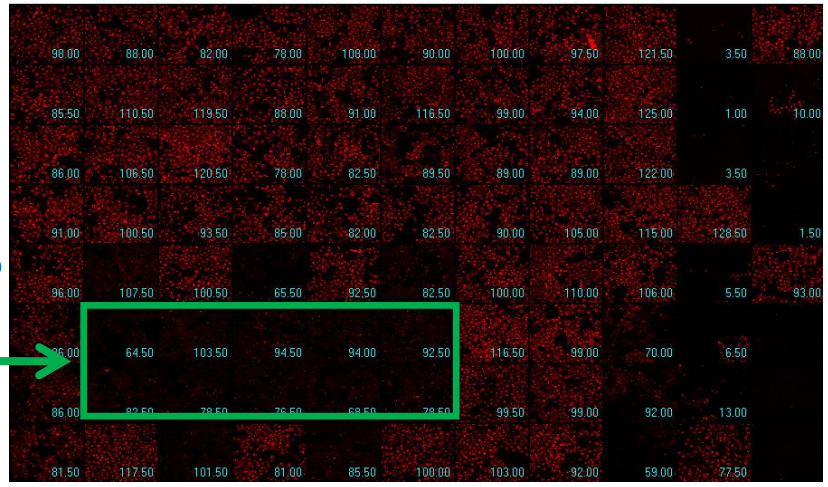


96-well view

Cells are present



Endocytosis reduced



We progress compounds that block both: In vitro and in-cell

Medicinal Chemistry Capability

Biotage Alstra

Our investment in accelerating compound access and scale up

- High quality synthesis; better yield, less variability
- Rapid method development or reaction optimization with low reagent usage
- Batch to flow to batch capability saves time and material
- Can couple ***MULTIPLE*** flow reactors / chemistries

Syrris ASIA / FRX

Biotage

Current Flow Capability

Homogeneous catalysis
- Suzuki reaction
- Heck reaction
- Grubbs ring forming

Multicomponent reactions
- Passerini 3CR
- Biginelli 3CR
- Ugi 4CR

Deprotection chemistry
- BOC deprotection
- MOM deprotection and intra epoxide opening
- Ester saponification

Ring formations
- Grubbs ring forming
- Ugi to benzimidazole
- Diels Alder
- 1,3,4 Oxadiazole formation
- Fischer indole synthesis
- 1,3 Thiazole formation
- Pyrazole formation

Oxidations and reductions
- Borohydride reduction
- Borane reduction of a heterocycle
- Reductive amination
- Dess Martin alcohol oxidation

General Synthesis
- Aldol reaction
- Biphasic Schotten-Baumann
- HBTU amide coupling
- Elimination of an alcohol to alkene
- Esterification of an alcohol
- Wittig reaction
- Nucleophilic aromatic substitution
- S_N1 reaction
- Mitsunobu reaction
- N-Alkylation

Heterogeneous capability with current systems

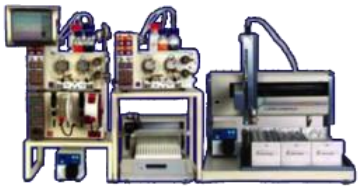
Anton Parr Monowave

VapourTec easy-Photochem

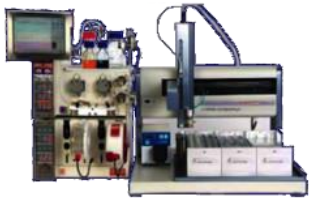
X-Cube

H-Cube / CartChanger

H-Cube Pro



VapourTec R2+; 4 Pump



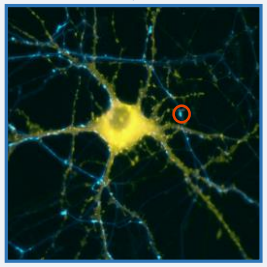
VapourTec R2S; 2 Pump

VapourTec easy-Medchem

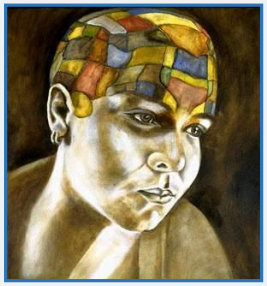
Clinical Translation of Targeting Endocytosis

Endocytosis in the brain / cord

Dynamin I



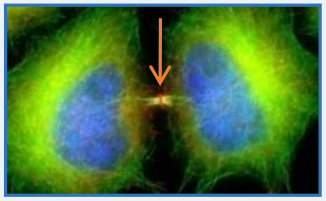
Synaptic transmission run-down



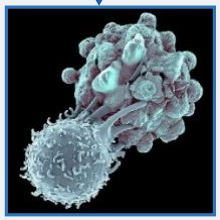
Epilepsy / Pain

Endocytosis in cancer

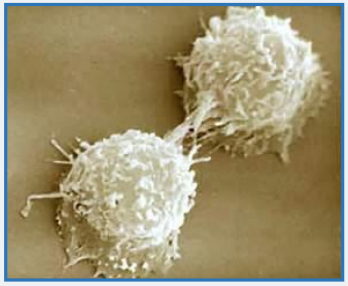
Dynamin II



Cell division failure (monotherapy)



Endocytosis block (combination)

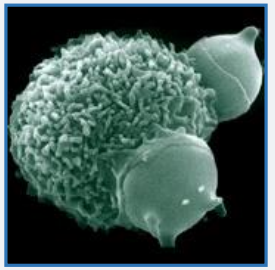


Cancer

Endocytosis in the body

Dynamin II

Pathogen/virus entry blocked



Infections

Acknowledgements

- Phil Robinson
- Adam McCluskey
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- **Ainslie Whiting**
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NHMRC

Cancer Council NSW

NATIONAL BREAST CANCER FOUNDATION

Australian Government
Australian Research Council

NSW GOVERNMENT

cancer institute NSW

Equipment funding

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