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Lohning, Anna Elizabeth; Marx, Wolfgang; Isenring, Elisabeth

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In silico analysis of the interactions of ginger actives with the serotonin (5-HT$_3$) receptor

Presenter: Asst/Prof. Anna Lohning
Faculty of Health Sciences & Medicine
Bond University
Gold Coast, Australia
**In silico** analysis of the interactions of ginger actives with the serotonin (5-HT₃) receptor

_Lohning, Anna E., Marx, Wolfgang_

**Clinical study:** Ginger as an effective anti-emetic agent for use in chemotherapy

Marshall, S., McCarthy, A., McKavanagh, D., Vitetta, L., Sali, A., Lohning, A., Marx, W., Crichton, M., Reid, K., Isenring, E.
Presentation Overview

• Rationale
• Background / Aim
• Methods
• Results
• Conclusions /
• Planned work
Rationale

• Chemotherapy-induced nausea and vomiting (CINV) poses a major obstacle to patients. Variable responses to current treatments for CINV reduces their effectiveness providing impetus to develop more effective treatments (Hsieh, 2015).

• Clinical trials have shown preliminary support for the use of ginger in multiple types of nausea (motion, morning sickness, chemotherapy-induced) (Marx, 2013).

• A key finding from a double-blinded, randomized-controlled trial (Marx, 2017) in chemotherapy-naïve patients was that intervention participants reported significantly better CINV-related quality of life (QoL) & less fatigue than placebo participants (Marx et al 2017).
Rationale (cont’d)

• In conjunction with the ongoing clinical studies, we’re interested in the mechanistic aspects of how ginger may function as an anti-emetic.

• *In vitro* studies have shown the active compounds in ginger
  a) Inhibit *serotonin (5-HT₃)-induced contractions* in guinea pig ileum¹
  b) Inhibit *serotonin-mediated signalling* (possibly in a non-competitive manner)²

• Current anti-emetic treatment for *CINV* (eg granisetron) *target 5-HT₃ receptors*

• Understanding the details of how ginger actives bind and interact with this receptor will help guide the design for *more effective treatments.*

---

² Walstab, J.et al Neurogastroentereol. Motil., 25 (2013) 439-447 (e302);
Introduction

• A primary pathway for emesis relating to **CINV** is stimulation of the vagal afferent nerves causing release of high levels of serotonin (5-HT$_3$)

• Serotonin binds to receptors on afferent nerves sending a signal to the central nervous system, mediating a range of physiological functions.

• Current treatment for **CINV** involves use of anti-emetics (**setrons**) that **competitively inhibit** 5-HT$_3$ receptors thus decreasing 5-HT response.

Serotonin

5-HT release

Stimuli includes luminal distension, parasympathetic innervation or changes in osmotic concentrations in intestinal contents.

ECCs = enterochromaffin cells
The 5-HT$_3$ subtype of serotonin receptors are cationic, *pentameric ion channels*. Other examples of this receptor type include GABA, glycine, nACh receptors.

5 distinct subunits (5-HT3$_{A\rightarrow E}$) leads to **complexity of function**. (eg Zn$^{2+}$ & small alcohols effect functional state of receptor.

Functionally, the channel can be either open, closed or desensitized – serotonin binds with high affinity to the **open** channel but
Introduction (cont’d)

• Gingerols are the primary bioactives within the non-volatile, pungent component of the ginger rhizomes (Zingiber officinale).

\begin{align*}
\text{Gingerols (n=6,8,10)} & \quad \text{Shogaols (n=6,8,10)} & \quad \text{Dehydoshogaols (n=6,8,10)} \\
\end{align*}

• *In vitro* studies by Abdel Aziz in 2005 found that 6S, 6G, 8G and 10G inhibited 5-HT_{3}-induced contractions of the isolated guinea-pig ileum.

• Since they were unable to displace ^3^HGR65630 (a competitive inhibitor) a non-competitive mechanism was proposed (potential allosteric site) Similar findings were reported by Walstab in 2013.

• However the mechanism remains unclear\textsuperscript{1,2}

Aims

1. Given there is no ligand-bound crystal structure to date, we aim to probe the serotonin and proposed allosteric sites with a range of in silico techniques that may suggest ginger actives may play a role at the 5-HT₃ receptor.

2. To compare the stability of 6-gingerol, serotonin and granisetron in each site using molecular dynamics simulations.
Method (Part 1):

**Target preparation**

- Homopentameric mouse 5-HT\textsubscript{3} receptor (4pir.pdb)
- Both serotonin & allosteric sites are located at interface of two subunits (principle/complementary) with key interacting residues from both subunits (A\textsubscript{P}A\textsubscript{C}) extracted
- 2 subunits (A\textsubscript{P}A\textsubscript{C}) extracted for analysis (ECM/TM/ICD) *
- Energy minimized (Gast-Hückel charges & H added)

* SYBYLx2.1.1 molecular modelling software
Method (Part 1):

**Ligand database preparation**

- Structures obtained either from Pubchem/PDB databases or prepared in ChemDraw.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin, (5-HT)</td>
<td>cognate ligand</td>
</tr>
<tr>
<td>6,8,10-G</td>
<td>Gingerols</td>
</tr>
<tr>
<td>6,8,10-S</td>
<td>Shogaols</td>
</tr>
<tr>
<td>6,8,10-DHSG</td>
<td>Dehydroshogaols</td>
</tr>
<tr>
<td>capsaicin, curcumin</td>
<td>Structural analogs of ginger actives</td>
</tr>
<tr>
<td>granisetron, ondansetron, etc</td>
<td>Positive Controls (5-HT site) (Setrons)</td>
</tr>
<tr>
<td></td>
<td>(competitive)</td>
</tr>
<tr>
<td>PU02, bicurculline, etc</td>
<td>Positive Controls (allosteric site)</td>
</tr>
<tr>
<td></td>
<td>(non-competitive)</td>
</tr>
<tr>
<td>Acetylcholine, GABA</td>
<td>Negative Controls (Decoys)</td>
</tr>
</tbody>
</table>

* Energy minimization Protocol

<table>
<thead>
<tr>
<th>Forcefield</th>
<th>Amber FF99</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amber atom types</td>
<td></td>
</tr>
<tr>
<td>Charges</td>
<td>Gasteiger-Huckel</td>
</tr>
<tr>
<td>Method</td>
<td>Steepest Descent</td>
</tr>
<tr>
<td>Convergence</td>
<td>0.5 kcal/mol</td>
</tr>
</tbody>
</table>
Molecular Docking (Surflex-Dock 2.1)

- Protocol: Serotonin site (multi-channel) Allosteric site (residue-based)
- “Flexible” docking approach (ligand & protein atoms around site of interest).
- Poses ranked according to Total Score (1/K_d) loosely approximating a theoretical binding affinity.
- C-score validation. Compares 4 scoring functions each with different weightings for non-bonded interactions.

![Diagram](image-url)

**Method (Part 1):**

serotonin site (A)                      allosteric site (B)

Protomols coloured by lipophilicity
Method (Part 1):

2. **GRID Analysis**

   - Interaction energies calculated at each grid point (kcal/mol) (Goodford, 1985).
   - Grid box (dimensions \((topx, y, z; botx, y, z)\)) generated around each site. (0.33 Å resolution)
   - A set of small atomic/molecular probes was selected to mimic the chemical properties of key functional groups of the ligands.

3. **Sequence Alignment**

   - ClustalOmega alignment between mouse and human 5-HT\(_3\) receptor sequences was performed to identify the degree of homology and identify conservation of residues likely to be important in ligand binding.

### Table: GRID for serotonin site

<table>
<thead>
<tr>
<th></th>
<th>Serotonin Site</th>
<th>Allosteric Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom</td>
<td>144.82</td>
<td>138.06</td>
</tr>
<tr>
<td>Top</td>
<td>181.15</td>
<td>184.06</td>
</tr>
<tr>
<td>Y</td>
<td>157.57</td>
<td>166.93</td>
</tr>
<tr>
<td>y</td>
<td>193.90</td>
<td>209.93</td>
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<tr>
<td>Z</td>
<td>231.82</td>
<td>250.75</td>
</tr>
<tr>
<td>z</td>
<td>277.82</td>
<td>293.75</td>
</tr>
</tbody>
</table>
Method (Part 2):

Molecular Dynamics Simulations

• Target Preparation
  • Initially a solvated dimer (A\(\_\_A\_\_\_\_\)), ECD domain in dodecahedron box (SPC water)
  • Gromacs-5.04 (FF – gromos54a7_FF – gromos54a7)

• Ligand Preparation
  • Topologies obtained from ATB\(^1\) & superimposed onto docked ligand pose.

• Usual preparation prior to full MD production run
  • EM (steepest descent, 1000 steps, conv.
  • NVT ensemble - Canonical isothermal thermostat (Berendson temp coupling)
  • NPT ensemble - barostat
  • MD – 10ns, 2fs ts


Key MDP Parameters
Neighbour coupling (Verlet) E’tatics
(Reaction-field, epsilon = 78)
Results – Molecular Docking

Serotonin site

- GRID analysis & Connolly surface (top) show lipophilicity nature of serotonin site.
  - **orange contours** (GRID 1.5 kcal/mol, strong interactions with hydrophobic probe)
  - **Serotonin** (total score 5.7) and **10G** (total score 10.81) docked into the serotonin binding site.
  - Top scoring 10G (& all other ligands) docked into a location **distinct and more hydrophobic** than that of serotonin.
  - Residues previously thought to be important for binding serotonin: **S176, R65, D42**
  - Additional residues found to interact with sertrons and ginger compounds: E173, D177 (E209 (granisetron))

- Position of key residues forming ‘aromatic box’ (**Y207, W156** P subunit; **Y127, W63** C subunit)
Results:

Sequence alignment (ClustalOmega)

• subunits A and B of the mouse & human 5-HT\textsubscript{3} receptors

• Key residues highlighted for :
  • principle subunit (blue shaded box)
  • complementary subunit (grey shaded box)
  • pore-facing residues of TM2 (red star *)
  • TM regions M1-M4 (underlined).

• Results show human & mouse 5-HT\textsubscript{3A} share ~85% sequence homology while 5-HT\textsubscript{3B} share ~73%. Human A & B subunits share only ~44%.

Key Finding:
All residues required for stabilising ginger compounds in both sites were conserved between mouse & human

FASTA colouring scheme (based on residue type)
• **Serotonin** scored mid field in both sites (polar)
• Ginger compounds scored high in both sites (as did structural analogs (all amongst most hydrophobic)
• **Competitive antagonists** scored mid field at both sites (very similar clogPs)
• **Polar non-competitive antagonists (NCAs)** scored lowest in serotonin site. The more lipophilic NCAs scored higher in serotonin site. (Nb. allosteric modulators are more potent in heteromeric receptors)
• **Decoys** (highly polar) scored poorly in both sites. (Most polar scored mid range in allosteric site)

Polarity was a key factor for binding in serotonin site than the allosteric site
Results – Molecular Docking

Serotonin site

• Ligand flexibility played a more important role than PSA in scoring
• Compounds scored high that were:
  - flexible & hydrophobic

Allosteric site

• Ligand lipophilicity (clogP) & flexibility / size were positively correlated.
• Compounds scored high that were:
  - flexible & hydrophobic
  - larger and hydrophobic
Results – Molecular Docking

Serotonin site

• Our results confirmed the importance of key residues thought to stabilise serotonin in this site, especially R65, N101, T154.

• Our results identified novel interactions with serotonin (D177, E173) and dolasetron (E209) and gingerols (K211, E209, L157).

• GRID successfully predicted position of aromatic ring of docked ginger actives.
Results – Molecular Docking

- Serotonin site – total scores ranged from 4.25-10.81 (-logK_D)
  - 10G scored highest (ginger actives & structural analogs scored highly)

- Allosteric site – total scores ranged from 3.94-9.23 (-logK_D)
  - Capsaicin (structural analog) scored highest followed by gingerol compounds in *allosteric site*

- Experimental IC_{50} data (where available) included for comparison with docking scores for highest binding pose/ligand.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1C_{50}</th>
<th>Total Score (logK_D)</th>
<th>Cascore</th>
<th>Hbonds</th>
<th>Interacting Residues</th>
<th>Total Score (logK_D)</th>
<th>Cascore</th>
<th>Hbonds</th>
<th>Interacting Residues</th>
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<tbody>
<tr>
<td>Serotonin Site</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6G</td>
<td>30 μM (at)</td>
<td>8.7</td>
<td>1</td>
<td>3</td>
<td>E209 R65</td>
<td>8.26</td>
<td>1</td>
<td>4</td>
<td>E219 Q56 F222 E53</td>
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<tr>
<td>8G</td>
<td>μM range</td>
<td>10.25</td>
<td>5</td>
<td>4</td>
<td>T154 E209 R65</td>
<td>8.84</td>
<td>5</td>
<td>3</td>
<td>E53 R219 F222</td>
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<tr>
<td>10G</td>
<td>μM range</td>
<td></td>
<td>4</td>
<td>5</td>
<td>T154 E209 K211 T152</td>
<td>8.26</td>
<td>1</td>
<td>5</td>
<td>T280 H139 E53 Q56</td>
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<tr>
<td>6S</td>
<td>9.3 μM (at)</td>
<td>8.31</td>
<td>0</td>
<td>2</td>
<td>N101 W156</td>
<td>6.52</td>
<td>0</td>
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<td>E53 F222 Q56</td>
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<tr>
<td>8S</td>
<td>μM range</td>
<td>9.06</td>
<td>5</td>
<td>4</td>
<td>R65 S155 T154</td>
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<td>2</td>
<td>K54 F222</td>
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<tr>
<td>10S</td>
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<td>9.34</td>
<td>2</td>
<td>2</td>
<td>T152 N101</td>
<td>8.29</td>
<td>5</td>
<td>1</td>
<td>F222</td>
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<tr>
<td>6DHS</td>
<td>-</td>
<td>6.97</td>
<td>0</td>
<td>3</td>
<td>T152 N101 K211</td>
<td>6.28</td>
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<td>3</td>
<td>E53 Q56 K54</td>
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<tr>
<td>8DHS</td>
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<td>8.56</td>
<td>0</td>
<td>3</td>
<td>L157 N101 Y207</td>
<td>6.61</td>
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<td>E186</td>
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<tr>
<td>10DHS</td>
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<td>2</td>
<td>2</td>
<td>L157 N101</td>
<td>6.85</td>
<td>4</td>
<td>3</td>
<td>E53 Q56 K54</td>
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<tr>
<td>Allosteric Site</td>
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<tr>
<td>Structural Analogues of ginger actives</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsaicin</td>
<td>-</td>
<td>8.54</td>
<td>0</td>
<td>4</td>
<td>R65 N101</td>
<td>9.23</td>
<td>1</td>
<td>3</td>
<td>K54 R219 F222</td>
</tr>
<tr>
<td>Curcumin</td>
<td>-</td>
<td>8.77</td>
<td>0</td>
<td>9</td>
<td>R65 T154 S155 D177 S179</td>
<td>7.02</td>
<td>0</td>
<td>3</td>
<td>R219 E53 E186</td>
</tr>
</tbody>
</table>

Residues in blue (previously suggested by Hassaine to be important for stabilising serotonin
Results – Molecular Docking

• The setron family of anti-emetics ranked mid-field at both sites

• Non-competitive ligands scored poorly as did decoys. (Nb. Allosteric ligands are observed to be more potent towards heteromeric targets)

• Cscores were high for 10G indicating a consensus between scoring functions for their overall ranking.

• Cscores were similarly high for serotonin, some setrons & non-competitive ligands.
Results: Molecular Docking

**Allosteric site**

Allosteric modulation permits fine-tuning of ion permeation via signal dampening.

The larger volume allows gingerols to adopt a more extended conformation facilitating favourable hydrophobic interactions with the transmembrane region.

![Molecular Docking Image](image)

Picrotoxin (NCA) is able to differentiate between A & B subunits¹.

---

Results: Molecular Docking
Allosteric site

Top scoring ligand, **capsaicin**. Ginger actives also score well. This site was found to be more hydrophobic compared to the serotonin site.

(A): GRID contours for a hydrophobic probe (-0.5 kcal/mol).

(B): Water probe (-11 kcal/mol) coincides with polar groups.

Connolly surface coloured by lipophilic character.
Results: Molecular Docking

Allosteric site

- Ginger actives ranked highly.
  - Gingerols > shogaols > DHSGs
- Order correlates with the higher polarity of the site.
- Unlike serotonin site, polarity was not the key determinant contributing to score
  - Eg. PU02 (clogP similar to ginger actives) scored low)

Key Finding:
Flexibility and hydrogen bonding capacity played a key role in binding interaction
Potential Energy over 10ns simulation

Serotonin Site

Allosteric Site
RMSD Comparison of Ligand Stability

Serotonin Site

Allosteric Site
<table>
<thead>
<tr>
<th></th>
<th>Serotonin Site</th>
<th>Allosteric Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>serotonin</td>
<td><img src="image1" alt="serotonin" /></td>
<td><img src="image2" alt="serotonin" /></td>
</tr>
<tr>
<td>6-gingerol</td>
<td><img src="image3" alt="6-gingerol" /></td>
<td><img src="image4" alt="6-gingerol" /></td>
</tr>
<tr>
<td>granisetron</td>
<td><img src="image5" alt="granisetron" /></td>
<td><img src="image6" alt="granisetron" /></td>
</tr>
</tbody>
</table>

**Trajectories**

- Serotonin Site: Images showing the interactions of serotonin with different molecules.
- Allosteric Site: Images showing the interactions of 6-gingerol and granisetron with different molecules.
Conclusion from MD analysis ... to date
Limitations

• Species differences

• Functional State

• Subunit Composition

• Transmembrane/ECM interface - another potential binding site

• Inherent in Molecular Docking approaches are
  • Inaccuracies in the energy models used to score potential ligand/receptor complexes
  • The inability of current methods to account for conformational changes that occur during the binding process not only for the ligand, but also for the receptor (i.e. how to cope with protein flexibility (1000’s of degrees of freedom)
  • The above can be alleviated by using the more robust, Molecular Dynamics (full protein flexibility) – see later.
Conclusions / Future Directions

Key Findings

- Serotonin bound to a site distinct from other ligands in serotonin site. This correlated with site hydrophobicity.
- Ligand hydrophobicity directly correlated to higher scoring in serotonin site while ligand flexibility and hydrogen bonding capacity facilitated more potent interactions at the allosteric site.
- Our results were in agreement with a number of key residues involved in stabilising serotonin (R65, N101 & T154) at the orthogonal site. Novel residues (E102 & R219) could be exploited in drug design.
- At allosteric site, novel residues, R219, Q56, F222, Q53 and I139 were important in stabilising ginger actives.
- Ginger compounds scored highly in both sites.
  - Structural characteristics (flexibility, hydrophobicity, Hbond acceptors/donors) enable them to exploit complementary features in a binding pocket. Similar dual roles have been observed.
Conclusions / Future Directions

**Analytical analysis**
Quantification of ginger actives was conducted in a range of commercial ginger products to determine (Marx et al, 2016).

**Future Work in Progress**
Clinical: - A larger clinical trial has been accepted for funding (NHMRC, Feb 2017).
Mechanistic: - MD for pentameric ion channel in membrane.
Research Team
Professor Liz Isenring
Head of Program, Nutrition & Dietetics
Research Group
Bond University, Gold Coast, Australia

Dr. Wolfgang Marx
School of Allied Health,
LaTrobe University, Melbourne, Australia

Alexandra McCarthy, Princess Alexandra Hospital, QLD, Australia, Division of Cancer Services, Institute of Health and Biomedical Innovation, Brisbane, QLD, Australia; School of Nursing, University of Auckland, Auckland, NZ.

Karin Ried, Research Director, National Institute of Integrative Medicine

Dan McKavanagh, School of Pharmacy, The University of Queensland, Brisbane, QLD, Australia; School of Nursing, University of Auckland, Auckland, NZ.

Luis Vitetta, Medlab Clinical Ltd, Sydney, NSW, Australia/University of Sydney, Sydney Medical School, Sydney, NSW, Australia.

Avni Sali, National Institute of Integrative Medicine, Melbourne, VIC, Australia
Thank you!

Questions