Identification of Target Integrin Thyroid Hormone Receptors From Sea Urchins

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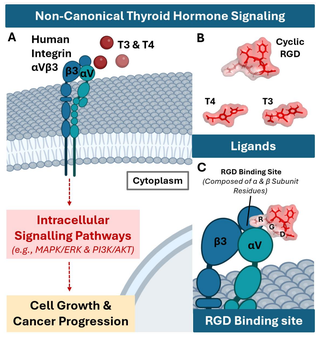
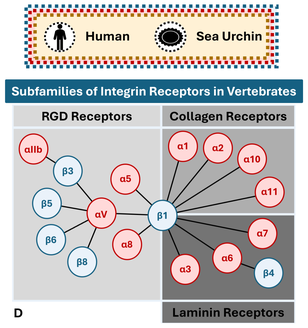
GitHub & References

1 Background

RGD Integrins, T3/T4 & Non-Canonical Signaling:

- Integrins are transmembrane heterodimeric receptors composed of one α and one β subunit. They mediate cell adhesion to the extracellular matrix (ECM) and facilitate bidirectional signal transduction across the membrane.
- Thyroid hormones (T3 and T4) regulate development, metabolism, and differentiation.
- Non-canonical thyroid hormone signaling (Panel 1A) involves membrane-associated receptors, (integrin αVβ3 in humans), which mediate rapid, transcription-independent responses.



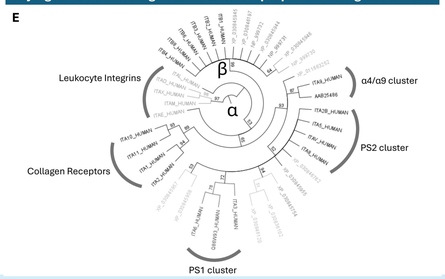


- RGD peptides (Panel 1B) are short amino acid sequences containing the Arg-Gly-Asp (RGD) motif that mimic natural ligands of integrin receptors.
- They bind to the RGD-recognition site (Panel 1C) on integrins such as αVβ3, which are often overexpressed in cancer cells, making RGD peptides targets and vehicles in cancer therapy.
- Vertebrates express 8 heterodimers that are RGD-binding (Panel 1D).
- The RGD integrin human αVβ3 also binds thyroid hormones and activates non-genomic signaling pathways involved in proliferation, angiogenesis, and tumor progression.
- While sea urchins lack a clear αVβ3 ortholog, thyroid hormones are known to bind sea urchin membrane proteins and accelerate larval skeletogenesis, suggesting non-canonical pathways may be conserved.

Knowledge Gap:

- Although sea urchins lack clear orthologs for some human integrins such as β3, phylogenetic analysis (Panel 1E) shows several S. purpuratus subunits cluster within conserved clades (e.g., PS1, PS2, α4/α9), suggesting potential functional analogs.
- However, the specific membrane receptors mediating non-canonical thyroid hormone signaling in echinoderms remain unidentified, highlighting a key knowledge gap.

Phylogenetic Clustering of Human and S. purpuratus Integrin Subunits



Hypothesis

I hypothesized that conserved or structurally analogous sea urchin integrin subunits form thyroid hormone binding pockets with varying selectivity for different thyroid hormone metabolites.

2 Tools & Methods

Six sea urchin αV-integrin heterodimers (αVβG, αVβ1-A, αVβC, αPβG, αPβ1-A, αPβC) were modeled and in-silico docking with key TH metabolites (T4, T3, TRIAC, TETRAC, sT3, sT4) was performed, using the human αVβ3 crystal structure as a reference.

2 Tools & Methods (cont'd)

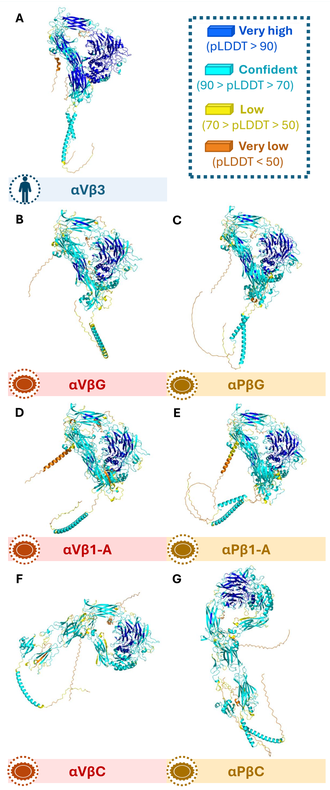
1. Docked RGD ligand to human αVβ3 (PDB 1L5G) as a proof-of-concept for the workflow.
2. Retrieved FASTA sequences of sea urchin integrin subunits from Echinobase and NCBI.
3. Modeled sea urchin integrin heterodimers using AlphaFold and chose the models with the best scores.
4. Modified PDB residue numbering and found equivalent key residues using ClustalW & Espript.
5. Obtained thyroid hormone metabolites from ZINC15 & PubChem, converting file types to PDB format using OpenBabel.
6. Docked each ligand to each receptor using HADDOCK and chose the best model from the best scored cluster. Pre & Post processing of files done using Python.
7. Obtained binding affinity scores (ΔG) for each HADDOCK model using PRODIGY.
8. Visualized structures in PyMOL for structural inspection and figure generation.

Main Tools Used:

- Echinobase & NCBI
- AlphaFold 3.0
- PubChem & OpenBabel
- ClustalW & Espript
- HADDOCK 2.5
- PRODIGY
- Linux & Python
- PyMOL

! Note: Although Mn^{2+} ions are biologically important for integrin activation and ligand binding, they were not incorporated into docking models.

3 Results & Discussion



AlphaFold Modeling of Integrin Heterodimers:

- AlphaFold-Multimer was used to predict the structures of one human (αVβ3) and six sea urchin (αV- and αP-containing) heterodimers using full-length α and β subunit sequences.
- Five models were generated per dimer, and the best model was selected based on interface predicted TM-score (ipTM), predicted TM-score (pTM), and per-residue confidence (pLDDT).
  - ipTM estimates the accuracy of the interaction interface between subunits with scores >0.7 suggesting a confident and well-packed interface.
  - pTM reflects the overall structural accuracy of the model with values >0.7 indicating reliable domain-level folding and global topology.

Receptor	Overall pLDDT	ipTM	pTM
αVβ3	83.28	0.83	0.84
αVβG	73.47	0.64	0.62
αVβ1-A	72.95	0.67	0.64
αVβC	71.30	0.63	0.60
αPβG	75.15	0.59	0.60
αPβ1-A	74.28	0.62	0.62
αPβC	72.55	0.58	0.60

Conclusions:

- The structure of human αVβ3 (Panel 3A) was confidently predicted, validating it as a high-quality reference for docking and comparison.
- Among the sea urchin integrins, αVβG (Panel 3B) and αVβ1-A (Panel 3D) had the highest interface confidence (ipTM = 0.64 & 0.67), suggesting potentially stable heterodimer formation.
- Other heterodimers (Panels 3C, E, F, G) showed lower ipTM/pTM values, indicating more uncertainty in dimer interface formation.
- Lower pLDDT scores were mostly outside the main extracellular domain, indicating reduced confidence in peripheral/flexible regions.

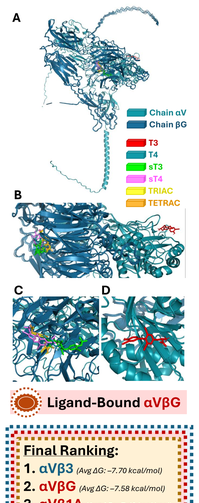
3 Results & Discussion (cont'd)

HADDOCK Docking of Ligands to Receptors:

- Docking was performed for each thyroid hormone metabolite with the human and sea urchin integrin receptors, with active residues defined for the predicted ligand contact regions.
  - Docking results were clustered by structural similarity, and the best model from the top-scoring cluster was selected based on HADDOCK score, Z-score, and favorable interaction energies.

PRODIGY Binding Affinity Scores: $\Delta G = RT \ln K_D$

- The ΔG (Binding Free Energy) of each HADDOCK model, which quantifies the energetic favourability of binding, was obtained.
  - More negative ΔG values indicate stronger, more favorable binding and less negative values reflect weaker or unstable interactions.
- The K_D (Dissociation Constant), which reflects the concentration of ligand at which half of the receptor is bound, was then calculated from the ΔG value (R = 1.987 cal/mol·K & T = 298.15 K).
  - Lower K_D values (nM–low μM) indicate strong binding and high affinity and higher values (>10 μM) suggest weaker or transient interactions.



Final Ranking:

1. αVβ3 (Avg ΔG: −7.70 kcal/mol)
2. αVβG (Avg ΔG: −7.58 kcal/mol)
3. αVβ1A (Avg ΔG: −7.49 kcal/mol)
4. αVβC (Avg ΔG: −7.47 kcal/mol)
5. αPβ1A (Avg ΔG: −7.44 kcal/mol)
6. αPβG (Avg ΔG: −7.06 kcal/mol)
7. αPβC (Avg ΔG: −6.91 kcal/mol)

Conclusions:

- Integrin dimers were ranked based on their average predicted binding free energy (ΔG) across six thyroid hormone ligands.
- Human αVβ3 showed the strongest overall binding (Avg ΔG: –7.70 kcal/mol), consistent with its known role in non-canonical thyroid hormone signaling in vertebrates.
- Sea urchin αVβG (Panels 3A-D) exhibited the next strongest overall binding with its ligands (Avg ΔG: –7.58 kcal/mol), suggesting it may be the closest analog of human αVβ3.
- In contrast, integrins containing the αP subunit (αPβ1A, αPβG, αPβC) showed weaker binding overall, implying they are less likely to mediate thyroid hormone signaling.

Binding Affinities (ΔG) & Dissociation Constant (kD) of Thyroid Hormone Metabolites to Receptors

Receptor	T4	T3	TRIAC	TETRAC	sT3	sT4
αVβ3	ΔG = –7.05 kcal/mol kD = 6.79 μM	ΔG = –7.35 kcal/mol kD = 4.09 μM	ΔG = –7.39 kcal/mol kD = 3.82 μM	ΔG = –7.38 kcal/mol kD = 3.89 μM	ΔG = –8.39 kcal/mol kD = 707 nM	ΔG = –8.61 kcal/mol kD = 488 nM
αVβG	ΔG = –7.30 kcal/mol kD = 4.45 μM	ΔG = –6.90 kcal/mol kD = 8.75 μM	ΔG = –7.75 kcal/mol kD = 2.08 μM	ΔG = –7.53 kcal/mol kD = 3.02 μM	ΔG = –8.31 kcal/mol kD = 809 nM	ΔG = –7.66 kcal/mol kD = 2.42 μM
αVβ1-A	ΔG = –7.40 kcal/mol kD = 3.76 μM	ΔG = –6.92 kcal/mol kD = 8.45 μM	ΔG = –7.52 kcal/mol kD = 3.07 μM	ΔG = –7.43 kcal/mol kD = 3.57 μM	ΔG = –8.23 kcal/mol kD = 926 nM	ΔG = –7.44 kcal/mol kD = 3.52 μM
αVβC	ΔG = –7.49 kcal/mol kD = 3.23 μM	ΔG = –6.73 kcal/mol kD = 11.7 μM	ΔG = –6.85 kcal/mol kD = 9.52 μM	ΔG = –7.68 kcal/mol kD = 2.34 μM	ΔG = –8.20 kcal/mol kD = 974 nM	ΔG = –7.87 kcal/mol kD = 1.70 μM
αPβG	ΔG = –6.37 kcal/mol kD = 21.4 μM	ΔG = –6.92 kcal/mol kD = 8.45 μM	ΔG = –7.21 kcal/mol kD = 5.18 μM	ΔG = –7.24 kcal/mol kD = 4.93 μM	ΔG = –7.65 kcal/mol kD = 2.47 μM	ΔG = –6.98 kcal/mol kD = 7.64 μM
αPβ1-A	ΔG = –6.69 kcal/mol kD = 12.5 μM	ΔG = –6.91 kcal/mol kD = 8.60 μM	ΔG = –7.47 kcal/mol kD = 3.34 μM	ΔG = –7.43 kcal/mol kD = 3.57 μM	ΔG = –7.91 kcal/mol kD = 1.59 μM	ΔG = –7.22 kcal/mol kD = 5.10 μM
αPβC	ΔG = –6.92 kcal/mol kD = 8.45 μM	ΔG = –6.73 kcal/mol kD = 11.7 μM	ΔG = –6.85 kcal/mol kD = 9.52 μM	ΔG = –7.09 kcal/mol kD = 6.35 μM	ΔG = –6.65 kcal/mol kD = 13.3 μM	ΔG = –7.21 kcal/mol kD = 5.18 μM

4 Acknowledgments

Apart from the support of my supervisors, I would like to thank Katherine Tieman for sharing her workflow, which guided my own analysis, as well as Dr. Dror Tobi for his guidance in configuring a primary computational tool.