Talin:Integrin Activation and Disruption

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Background

Hyperactivity of integrins can contribute to severe inflammatory responses, autoimmune diseases, and heart attack and stroke. Integrin cytoplasmic domains and talin forms an intracellular complex facilitating inside-out signaling to activate integrins, and is an important component of focal adhesions. Inhibiting integrin:talin interactions could serve as a novel, first in class approach to regulation of integrin function.

Table 1	2MWN	2H7E	3G9W	6VGU
FERM Domains	F3	F3	F2, F3	F0, F1, F2, F3
Binding Partner	RIAM	Chimeric β3 integrin & PIP2	β1 integrin	β3 integrin
Talin, Species	Talin1, Humans	Talin1, Gallus Gallus	Talin2, Mouse	Talin1, Mouse

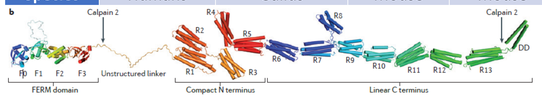
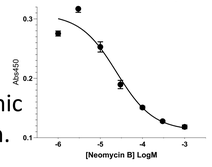


Figure 1: (Ref. 1) Structural representation of Talin.

In a separate study, we have identified aminoglycoside antibiotics, like Neomycin B, that inhibit integrin cytoplasmic domain interactions with talin.



Methods

Using MD simulations in GROMACS and docking with AutoDock Vina, talin conformations were generated to identify potential binding sites of candidate drugs. Our lab has experimentally validated at least 3 naturally derived products that have demonstrated some talin:integrin inhibition.

Table 2	Experiments	Status
2MWN	Apo	40 ns (my project)
	3 Poses w/Crystal Structure	40 ns (my project)
	3 Poses w/Added KKKKS Tail	40 ns & Docked (my project)
2H7E	ZINC	Docked 638,636 (Previous student)
3G9W	Apo	10 ns (Previous student)
	Neomycin B	Docked (PI)
6VGU	β3 Integrin Tail	Needs preparation
	Neomycin B Pose A	QM & Docked (PI)
	Neomycin B Pose B	QM & Docked (PI)

I am expanding the search for inhibitors using Neomycin B and known talin interacting partners like RIAM[(Ref. 5,6)]. Our Drug Discovery program is searching 15 mil. ZINC compounds for new leads.

Results

Figure 1: I added the poly-lysine motif to the end of 2MWN to properly prepare the structure for MD simulation runs (3 x 40 ns).

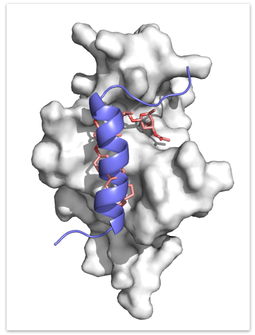


Figure 2: Highest affinity of structure 1 with Compound A with a ΔG of -9.845 kcal/mol. Docked as a potential competitive inhibitor

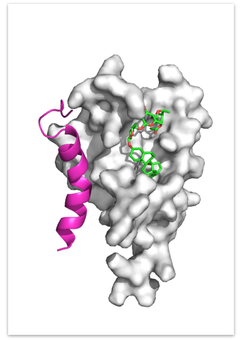


Figure 3: Highest affinity of structure 3 with Compound A (ΔG being -11.045 kcal/mol). Docked as a potential allosteric inhibitor

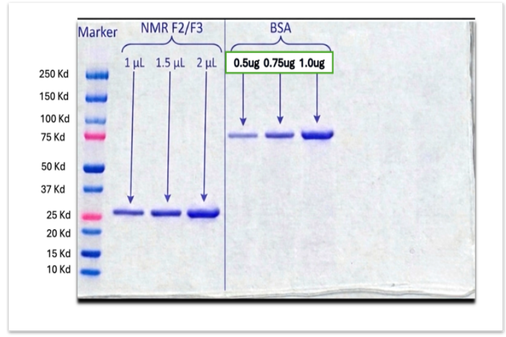


Figure 4: 15N-His-Talin-F2F3 grown in M9 minimal media (Anuj Kurella). This is foundational to confirm binding modes of leads generated during this project.

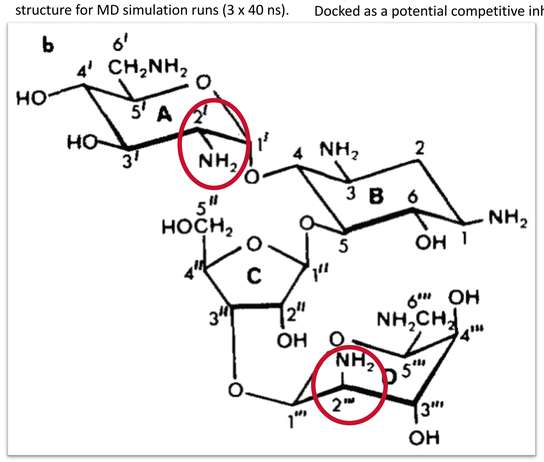


Figure 5: (Ref. 4) Structural representation of Neomycin B with the point of interest being 2′ NH_2 and 2‴ NH_2.

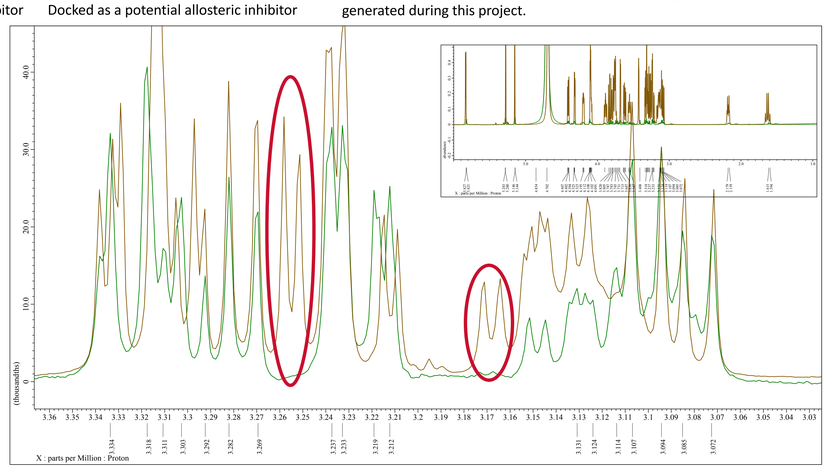


Figure 6: NMR spectra of Neomycin at two different pH levels, Green: ~8.5, and Brown: ~7.0. The brown peaks inside the red circle do not appear in the green spectrum, suggesting the point of interests were deprotonated at the higher pH.

```
# ============================== USER SETTINGS ==============================
THRESHOLD    = 0.75          # contact cut-off (nm)
FIRST_LIG    = 7
LAST_LIG     = 30
FILE_PREFIX  = "interaction_Prot"    # files: interaction_Prot<res>_Lig<N>.xvg
IN_DIR       = "."           # where the .xvg files live
OUT_DIR      = "."           # where PNGs are written
TIME_LABEL   = "Time (ns)"   # change to "Time (ps)" if that's your unit
SHARED_Y     = True          # True  -> every panel of a ligand shares one y-range
                             # False -> each panel autoscales its own y
MAX_LEGEND   = 14            # draw a legend only if a panel has <= this many lines
MAX_POINTS   = 4000          # down-sample any line longer than this (speed/size)
DPI          = 150
# ===========================================================================

plt.rcParams.update({
    "font.size": 10,
    "axes.titlesize": 12, "axes.titleweight": "bold",
    "axes.labelsize": 11,
    "axes.grid": True, "grid.alpha": 0.25, "grid.linestyle": ":",
    "axes.axisbelow": True,
    "legend.frameon": True, "legend.framealpha": 0.85,
    "figure.facecolor": "white", "savefig.facecolor": "white",
})


def residue_label(path, lig):
    """Pull the residue tag out of interaction_Prot<TAG>_Lig<N>.xvg."""
    b = os.path.basename(path)
    b = re.sub(r"\.xvg$", "", b)
    b = re.sub(r"^" + re.escape(FILE_PREFIX), "", b)
    b = re.sub(r"_Lig%d$" % lig, "", b)
    return b if b else os.path.basename(path)


def load_xvg(path, max_points=MAX_POINTS):
    """
    Robustly read (time, distance) from an .xvg file.
```

Figure 7: Autonomous Python code developed by Claude AI in response to a prompt to analyze data points from bonding interactions, as shown in **Figure 7.**

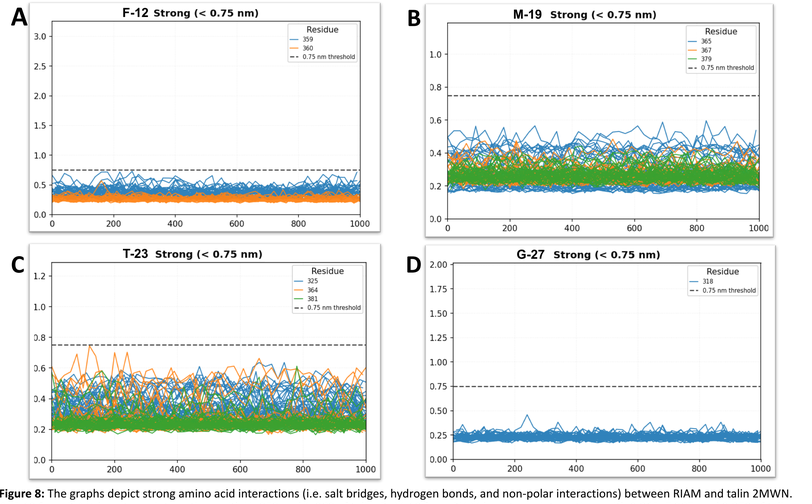


Figure 8: The graphs depict strong amino acid interactions (i.e. salt bridges, hydrogen bonds, and non-polar interactions) between RIAM and talin 2MWN. **A**: F-12 from RIAM has strong interactions with W-359 and A-360 of talin. **B:** M-19 from RIAM has strong interactions with S-365, T-367, and S-379 of talin. **C:** T-23 from RIAM has strong interactions with L-325, K-364, and Q-381 of talin. **D:** G-27 from RIAM has only one strong interaction with K-318 of talin.

Conclusion

Talin is a feasible target for drug design as demonstrated by its role in integrin signaling and mechanical coupling. Binding of compounds have been validated experimentally, and preliminary leads have been generated by computational drug discovery. We are poised to launch a large-scale composite discovery program that deploys computational chemistry, synthesis, and experimental validation in cell-free assays, cell-based assays, and in animal models. I have prepared ensembles of talin structures with side chain diversity for the expanded docking campaign. I have prototyped some of the AI components our team will use to automate the workflow. I collected NMR spectra of one of our binders (Neomycin B) at two pH levels to explore its protonation states. We are preparing isotope-labeled talin protein to evaluate NMR chemical shifts in the binding footprint of hit compounds.

References

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