Antibody-functionalized magnetic nanoparticles for translation of mechanosensitive receptor signaling in bone

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TRANSLATIONAL CHALLENGE

For mechanomedicine, the problem is how to control and apply forces remotely from outside the patient

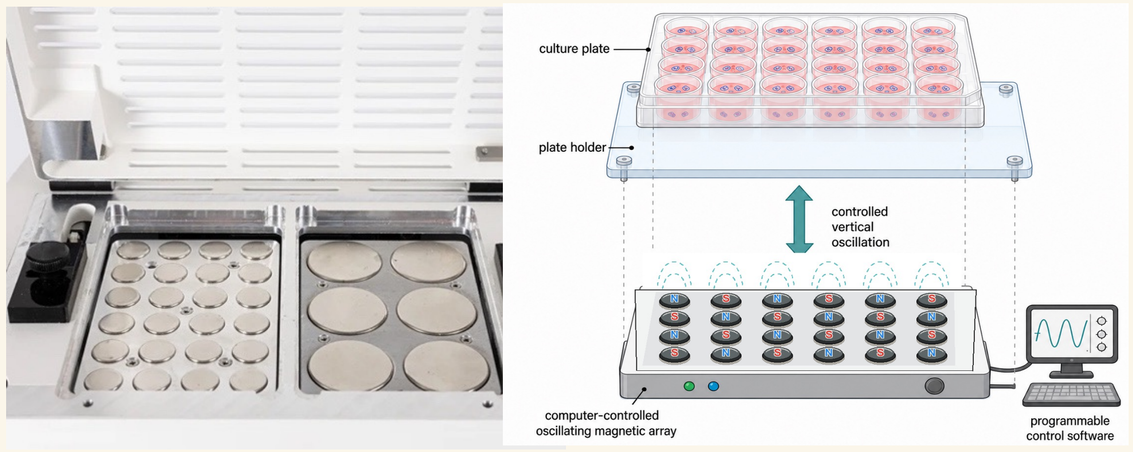
The aim is to generate:

localized force
at the particle–TREK1–cell interface

model-guided
Docking/MD narrows antibody choices

functional
Establishing longer term downstream pathways

Question: can modelling + magnetic actuation isolate TREK1-linked mechanosignaling in bone-regeneration cells?

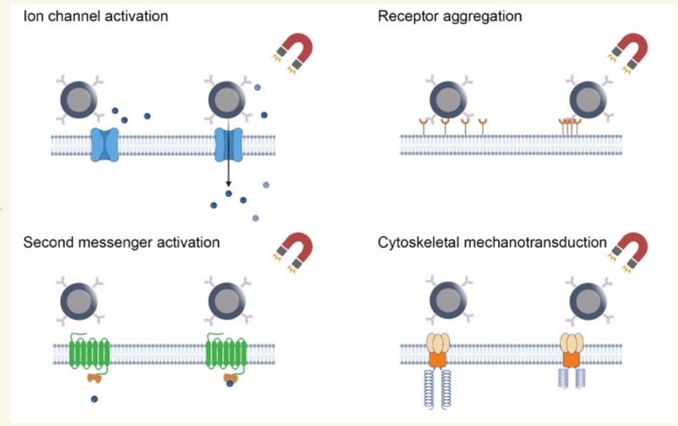


Magnetic force bioreactor / magnetic array platform used for remote stimulation studies

Modelling addresses which antibody can access TREK1 mechano binding sites; MICA then tests whether that model-selected interface can be mechanically actuated.

WORKING APPROACH

Magnetic ion channel activation (MICA)



Mechanism schematic adapted from Rotherham et al. (2022)

Core aim: build a mechanomedicine route from modelled TREK1 access → magnetic actuation → functional therapy.

HOW? One connected workflow, where modelling defines wet experimental validation

Direct activation is only interpretable when the model, particle behaviour, target validation and readout all agree.

01 Compare MNPs
TEM + DLS + Zeta potential before and after antibody functionalization in DPBS(pH=7.4 at RT)

Particle selection

02 Modelling
Model TREK1 antibody–channel binding: docking + MD + contact sites

03 Track iron uptake / degradation
ICP-OES for iron degradation from cell supernatant medium/ iron release from cell lysate with Y201 cell line

Model-guided validation

04 Validate TREK1 access
Immunofluorescence(IF) + Western blotting(WB) with MSC cell

Mechanism

05 Confirm function
Thallium-ion flux + controls With HEK293

MNPs:
MM250=dextran iron oxide composite particles with 250 nm

IOC116= citrate iron oxide particles with 116 nm

Antibodies:
Santa Cruz: TREK1 immunoglobulin M (IgM)

Twist Short/Twist Complete: TREK1 immunoglobulin M (IgM)

RGD = Arg–Gly–Asp peptide

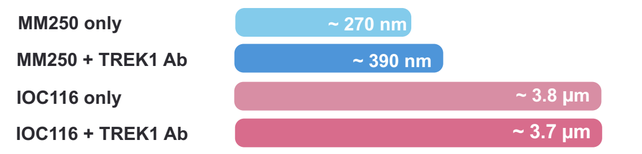
TREK1 = TWIK-related potassium channel 1. "Targeting" here means in vitro particle–cell/TREK1 access.

WHAT THE DATA SHOWS

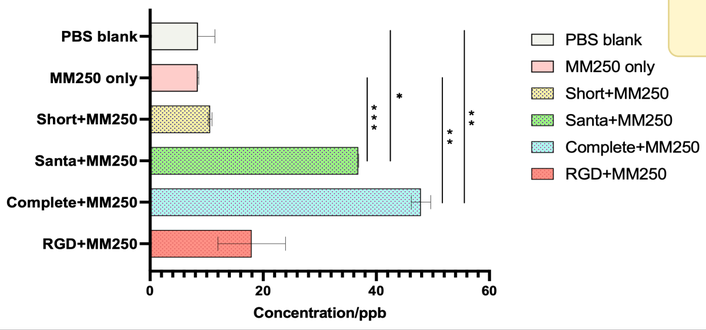
For mechanobiology in mechanomedicine, modelling idefines the next steps for experimental validation.

1 Selection of MNPs

Hydrodynamic diameter, surface charge and cytotoxicity define whether the biology can be interpreted.



MM250 / IOC116 = MNP formulation IDs • bars use a log scale



Surface charge after Santa Cruz TREK1 immunoglobulin M (IgM) functionalization

MM250 -7.1 mV → - 4.5 mV

IOC116 - 27.5 mV → - 23.7 mV

Antibody functionalization changes the interface in both MM250, but does not rescue aggregation for IOC116.

AT DAY 7

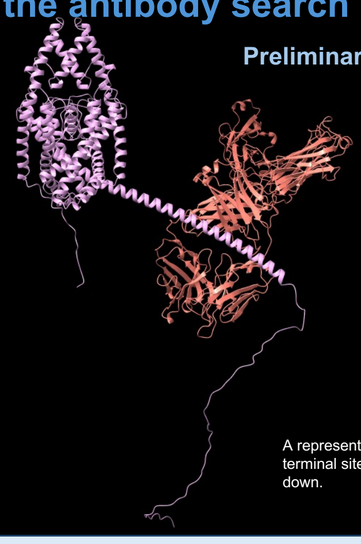
Highest iron degradation in DPBS :
MM250 + Santa Cruz;
MM250 + Twist Complete

ppb = parts per billion
Uptake and degradation in cells continue…

2 Modelling narrows the antibody search

- ✓ We docked candidate antibodies against TREK1 to find plausible binding poses.
- ✓ For each AlphaFold variant of the IgG and IgM candidates, we refined 10,000 models by energy minimisation, clustered them, and ranked them with PRODIGY.
- ✓ Alanine scanning on the top 10 clusters then picked out the interface residues that actually matter.

Preliminary TREK-IgG binding model



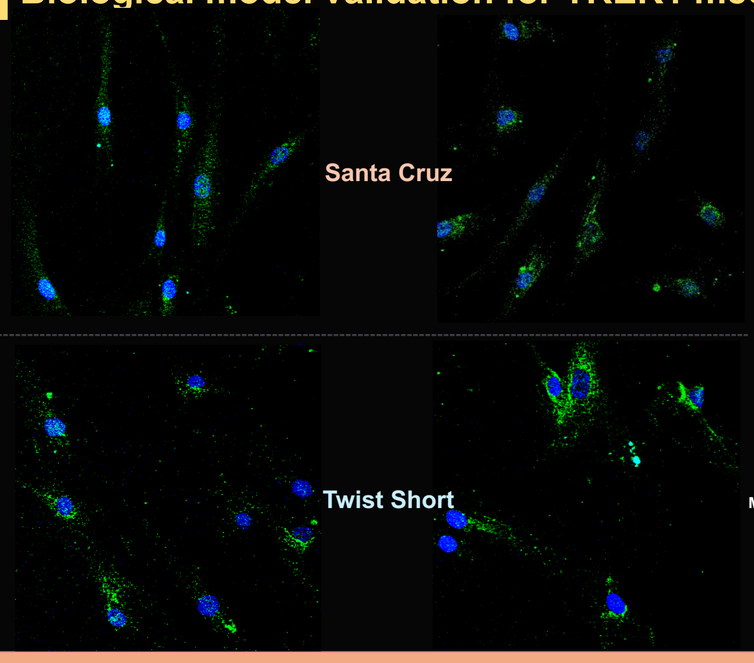
MODEL OUTPUT

Ranking gave us a short list of TREK1-binding poses to take into biological validation — a starting point for which antibodies to test first.

A representative pose: a candidate IgG on a possible TREK1 C-terminal site. We removed the N-terminus to keep the compute down.

Decision: take the top-ranked antibody–MNP candidates into IF/WB and ion-flux assays for testing.

3 Biological model validation for TREK1 mechano-activation



Immunofluorescence target validation current evidence and caution

DAPI: nuclear stain

Anti-TREK1-FITC: green channel 488

Santa Cruz gives the clearest current pericellular signal

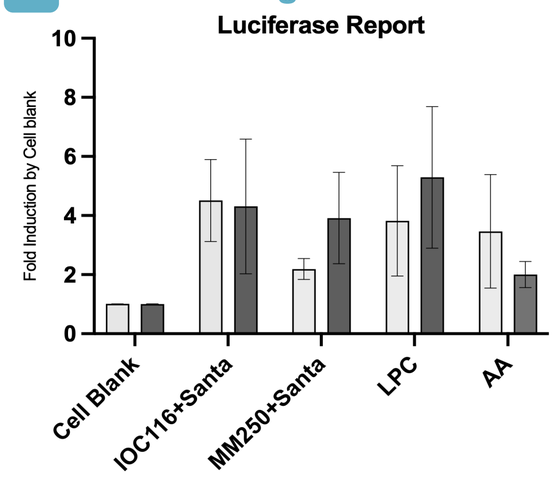
Twist Short also shows strong signal

Matched exposure • cell number • replicates

WB: candidate band near expected TREK1 size (~47 kDa); specificity still needs work.

Decision: Santa Cruz + Twist Short move forward only after matched IF exposure and WB specificity controls.

4 Establishing a TREK1 channel functional readout



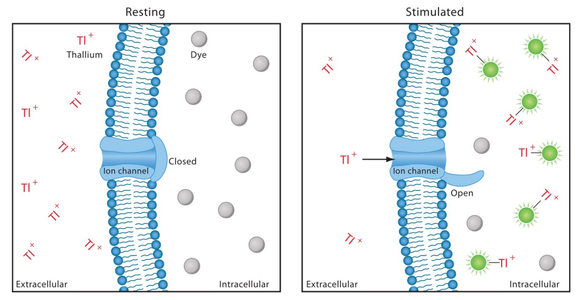
Luciferase readout was high for positive controls, MM250 showed elevation of the luciferase reporter but not IOC116. Both cases used the Santa Cruz antibody

Positive Control
AA = arachidonic acid
LPC = lysophosphatidylcholine

Next step will test TREK1 activity directly with ion flux.

NEXT: DIRECT TREK1 FUNCTION

We aim to measure thallium-ion flux across the membrane to demonstrate ion channel activity



Principle of the planned thallium-flux readout: when TREK1 opens, Tl^+ enters down its gradient and the FluxOR™ dye fluoresces — resting (closed, dim) vs stimulated (open, bright).

TO REMEMBER

01 MM250 provides the practical actuator
IOC116 aggregation limits functionality of the MNP as an actuator.

02 Modelling defines the best antibody site for binding
Docking, MD and accessibility turn antibody choice into a testable hypothesis.

03 Mechanomedicine requires function
Confirm TREK1 specificity and thallium-ion flux before antibody humanization.

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Thallium-flux schematic (Section 4) adapted from FluxOR™ Potassium Ion Channel Assay, Thermo Fisher Scientific (MAN0001946).

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