Biophysical Characterization of “Stapled” Single Chain Antibodies for Multispecific Biotherapeutics

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Tumor cells expressing ‘danger signal’, a mechanism for anti-cancer activity by natural killer cells

Credit: Xiefan Lin-Schmidt, Exploratory Biology, Therapeutics Discovery

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Increasing Emphasis on Multispecific Antibody Formats in Clinical Development and Discovery

Kaplon et al. 2023 https://doi.org/10.1080/19420862.2022.2153410

Biswas et al. 2023 https://doi.org/10.1080/19420862.2023.2207232
Increasing Reliance on Subcutaneous Delivery and High API Concentrations Challenges Multispecific Formats

Biswa et al. 2023 https://doi.org/10.1080/19420862.2023.2207232

Martin et al. 2023 https://doi.org/10.1080/19420862.2023.2191301
Many Novel Multispecific Antibody Formats Rely on Single Chain Antibody (scFv) Building Blocks

Engineering and optimization of scFv molecules, a 25-year-old problem:

New protein engineering approaches to multivalent and bispecific antibody fragments

A Plückthun, P Pack

Biswas et al. 2023 https://doi.org/10.1080/19420862.2023.2207232

Wörn & Plückthun, 1999. DOI: (10.1021/bi9902079)
scFv Containing Molecules Are Consistently More Prone to Aggregation Than Fab-based Molecules at Higher Concentrations

Stability after 40°C Incubation @ > 50 mg/mL

AUC

SEC

Janssen internal results
“Stapling” of scFv Fragments To Stabilize Against Concentration and Temperature Induced Aggregation

Hypothesis:

Solution: Stapled scFv

Design goals:

- Prevent scFv breathing and resultant aggregation
- Universal solution for all scFv molecules
- Retain biological potency and critical quality attributes
- No disulfide mispairing or unwanted side products
Location of Anchor Positions and Rigid Linker Region Designed To Prevent Disulfide Scrambling
Stapling Increases Conformational Stability Through Disulfide Bond Formation

Melting Temperature Increase by ~ 12 °C

Tm increases are independent of orientation and linker length

Structural Validation

Differential Scanning Calorimetry

<table>
<thead>
<tr>
<th>Molecule name</th>
<th>Orientation</th>
<th>scFv</th>
<th>spFv</th>
<th>∆Tm</th>
<th>∆∆H kcal/mol</th>
<th>spFv Linker Length</th>
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<tr>
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<td>71.6</td>
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</tr>
</tbody>
</table>
spFv Staple Forms a Consistent Structure Within Multiple Antibodies

spFv HL unbound (blue, green) vs. scFv LH bound (silver)

spFv HL unbound (blue, green) vs. spFv LH bound (silver)
spFv Forms Proper Disulfides in Bispecific Antibody Format
Bispecific Aggregation at High Concentrations is Alleviated by Stapling

150 mg/mL thermal stress

Viscosity 100 mg/mL:
scFv Bispecific = 3.6 cP
spFv Bispecific = 2.9 cP
Improvements to Aggregation Without Compromising Other Critical Attributes of a Bispecific

- Conformational Stability
- Charge Profile
- Surface hydrophobicity

PTM/Behavior under stress:
- Identical PTM profile by peptide mapping
spFv Retains Biological Potency of scFv Parent Bispecific Molecule

CD3 Binding

BCMA Binding

Cris7b Bird scFv x mAb1

Cris7b spFv x mAb1

Killing

% Quot. of total target

0.000001 0.0001 0.01 1 100

Concentration (nM)

CD4+ Activation

% Activated, of total CD4+ cells

0.000001 0.0001 0.01 1 100

Concentration (nM)

CD8+ Activation

% Activated, of total CD8+ cells

0.000001 0.0001 0.01 1 100

Concentration (nM)
Stapling Consistently Improves Thermal Induced Aggregation of scFv Molecules in Complex Formats

Stability after 40°C Incubation @ > 50 mg/mL

% Monomer (SEC)

- scFv Bi/Multispecific
- mAbs and Fab Bispecifics
- spFv Bi/Multispecific
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