Protein Engineering of Staphylokinase with Improved Thrombolysis

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Staphylokinase

- Activates plasminogen using plasmin, more fibrin-specific than alteplase
- Easy to produce, cheap, non-immunogenic variants available
- Non-inferior to alteplase for ischemic stroke treatment
- Potential to increase activity 1000-fold

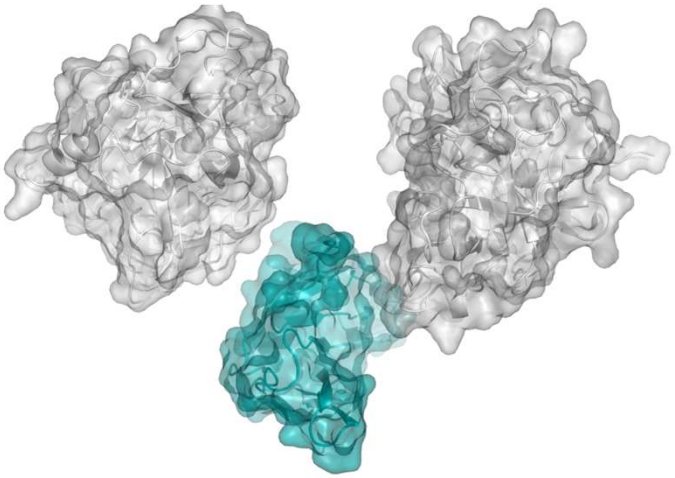

Figure 1: Staphylokinase (blue) complexed with plasmin (gray, left), which activates plasminogen (gray, right)

Computational Design for Improved Affinity

- Interface with „partner" plasmin designed using the AffiLib method
- Calculated binding energy of 18 thousand three to five-point mutants
- Best 50 binders: thermostability assessment: 14 stabilizing
- Four mutants (SAK01-SAK04) selected for production and testing

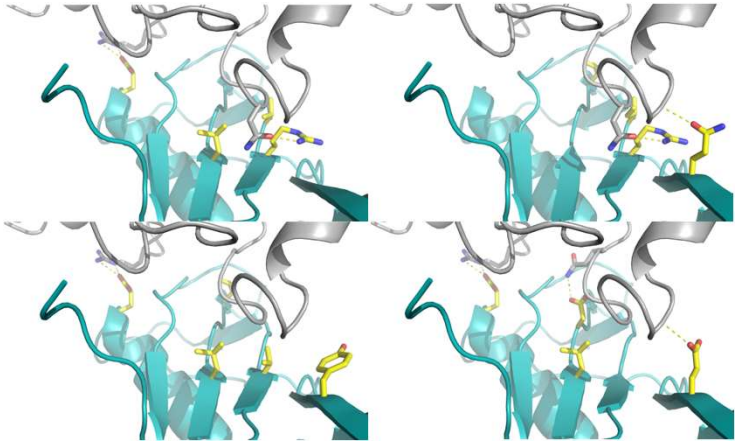

Figure 2: Interfaces of plasmin (gray) with staphylokinase mutants SAK01-SAK04 (blue, clockwise) with mutations (yellow) and polar contacts (dashed lines)

Improved Affinity and Selectivity

- Mutant SAK01 has shown 6-fold increased plasmin binding and 8-fold increased plasmin selectivity
- Binding towards plasminogen was unchanged or lowered
- SAK01 has shown 120% fibrinolysis rate of wild-type staphylokinase
- Good affinity was compensated by poor catalytic efficiency

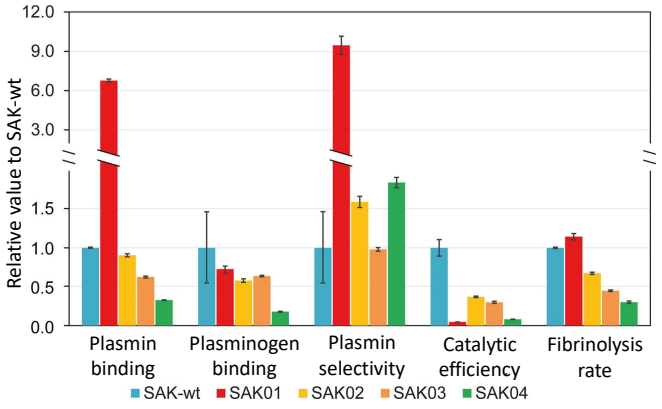

Figure 3: SAK01-SAK04 Mutant Characteristics. Binding and selectivity were measured by surface plasmon resonance, catalytic efficiency by equilibrium kinetics, and fibrinolysis using the fibrin plate method.

In Vitro Thrombolysis

- Static model: fibrin-rich semisynthetic and erythrocyte-rich healthy donor blood thrombi
- Dynamic model –stroke patient's CT-based circulation
- SAK01 is similarly effective to wild-type staphylokinase
- SAK01 and sak-wt have more effectivity and clot penetration than alteplase

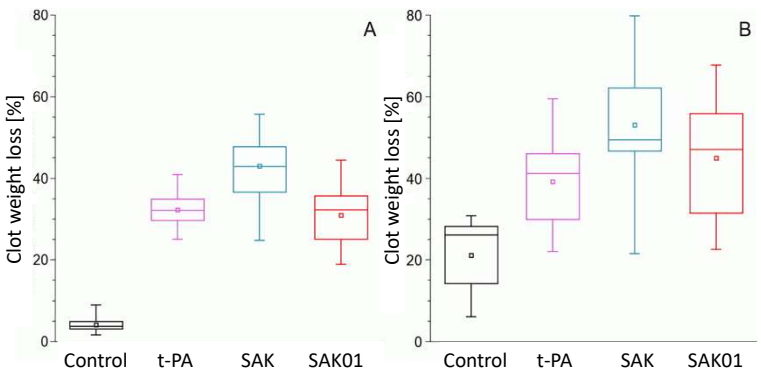

Figure 4: Clot weight loss of semi-synthetic (A) and erythrocyte-rich (B) thrombi. In the static lysis model of healthy donor's blood clots, SAK01 (red) is similarly effective to wild-type SAK and more effective than alteplase (t-PA, pink).

Conclusions and Perspectives

- SAK01 proved the concept of improving thrombolysis via affinity design
- We are currently researching staphylokinase and stroke treatment using:
  - Ribosomal display (high-throughput affinity design, millions of variants
  - In-house AffiLib calculations + machine learning analyses
  - Combinations of thrombolytics (SAK + alteplase, SAK + tenecteplase)

More Information

Clinical, biochemical, and biophysical references

Your feedback

Why can staphylokinase be a thousand times effective?

Our published works

https://muni.cz/go/staphylokinase

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