Calcium-Induced Activation of FXIII and Structure-Based Design of Direct Acting Inhibitors

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Introduction
Factor XIII represents a highly promising target for the development of novel anticoagulants with a lower bleeding risk compared to current anticoagulants. So far, all attempts to develop drug-like FXIIIa blockers failed. No compound entered clinical trials yet. This might at least be partly attributed to the lack of a crystal structure of FXIII in complex with an inhibitor to enable structure-based drug design. The crystal structure of FXIII in the inactive state, published in 1994, is not suitable because its active site is covered by the β-barrel 1 domain.

Materials & Methods
Purified recombinant human cFXIII was incubated with inhibitors in presence of calcium. A suitable crystalization condition was determined by performing a comprehensive crystallization screen. The binding mode of different peptidic inhibitors was analyzed at a molecular level.

Results
The crystal structure of FXIII in complex with the inhibitor reveals three calcium-binding sites whereas the two β-barrel domains flip aside and expose the active site. Calcium-binding affects the shape of the active site and triggers the formation of a catalytic dyad. Besides the already published structure of FXIIIa-ZED1301, we report another three crystal structures to decipher the binding mode in more detail. We showed that variation at the N-terminus of the peptidic inhibitors improved potency by one order of magnitude compared to the lead structure.

Conclusion
The crystal structure of FXIII in the active state provides mechanistic insights into the calcium-facilitated activation process and provides valuable information for the rational design of FXIIIa inhibitors.

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