A microfluidic approach for the directed evolution of proteins by retroviral display

Lucia Granieri, Jean-Christophe Baret, Andrew D. Griffiths and Christoph A. Merten

Institut de Science et d'Ingénierie Supramoléculaires, Université Louis Pasteur, 8 allée Gaspard Monge, 67083 Strasbourg Cedex, France.

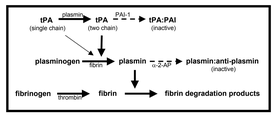
CNRS UMR 7006, 8 allée Gaspard Monge, 67083 Strasbourg Cedex, France.

Introduction

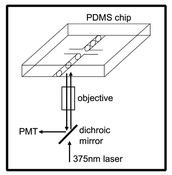
Phage display[1,2] is a widely used method for directed evolution of proteins, allowing the generation of an enormous diversity of protein variants displayed on the viral particles (library diversity $<10^{12}$). These protein variants can then either be selected for binding affinity (e.g. antibodies) or for catalytic activity (e.g. enzymes). However, since selection for catalytic activity requires immobilized substrates and/or products, selection for multiple turnovers or maximum rate acceleration remains challenging. To overcome these limitations a new method has been developed: Microfluidic-based compartmentalization of viral particles displaying single protein variants on their surface. Encapsulation of these particles into picoliter drops allows the use of soluble substrates/products and therefore the selection for multiple turnovers. The model system used here is based on retroviral particles displaying tPA, a protein used in current emergency therapies of myocardial infarction and stroke. Single tPA variants were encapsulated into aqueous droplets[3,4], at a frequency of ~10Kilohertz and the enzymatic activity was monitored using a fluorescence assay. Active variants could be clearly distinguished from inactive variants or variants incubated with the endogenous inhibitor PAI-1. The integration of a further sorting module should thus allow the specific selection of variants with high catalytic activity out of a diverse library.

Results

The physiological fibrinolytic system

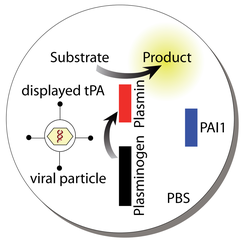


The optical setup



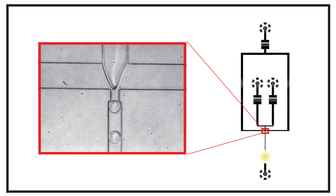
Viral particles were mixed on-chip with 5µM plasminogen and 3mM substrate. After 3 hours off-chip incubation, the fluorescence signal at 450nm showed a 100X increase.

The enzymatic reactions in a droplet

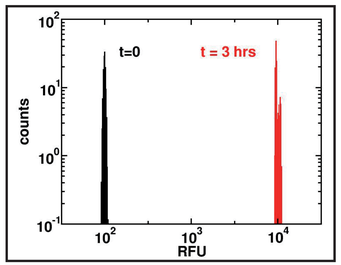


tPA was displayed on viral particles. tPA converts plasminogen into plasmin which in turn converts a fluorogenic substrate into a fluorescent product. This product shows an excitation/emission maximum of 370/450 nm.

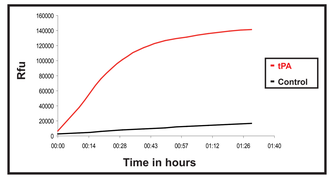
Mixing of viral particles, plasminogen and substrate



Microfluidic assay: Enzymatic reaction in droplets



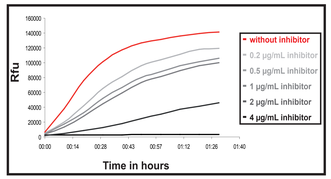
Viral particles displaying tPA or an unrelated control protein



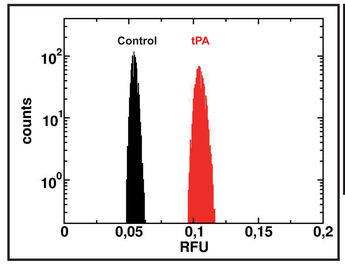
Viral particles displaying tPA showed an almost 10X higher fluorescence signal compared to the control (particles displaying the unrelated protein Neuraminidase).

Inhibition of the tPA by the endogenous inhibitor PAI1 resulted in a dose-dependent decrease of the fluorescence.

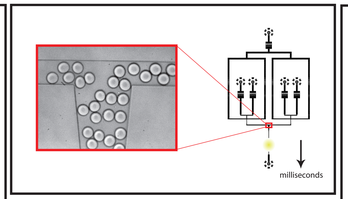
Viral particles in presence or absence of the endogenous inhibitor PAI1



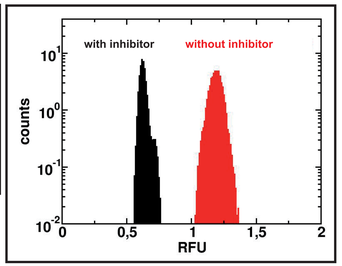
Viral particles displaying tPA or an unrelated control protein



Experimental setup for fluorescence detection



Viral particles in presence or absence of the endogenous inhibitor PAI1



Conclusions

Complex enzymes like tPA can be functionally displayed on retroviral particles.
The enzyme activity within drops can be determined quantitatively and decreases with increasing concentrations of endogenous inhibitor.
The encapsulation of particles in drops expands the application range of conventional techniques (e.g. phage display) and should allow selections towards enhanced enzyme activity.

References

[1] Smith et al., Science 1985, 228: 1315 - 1317, Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. [2] Winter et al., Nature 1991, 1352: 624 - 628, Making antibody fragments using phage display libraries. [3] Griffiths et al., Nature Methods 2006,3: 561 - 571, Directed evolution by in vitro compartmentalization. [4] Huck et al., Angewandte Chemie 2008, 120: 1997 - 2008, From microdroplets to microfluidics: selective emulsion separation in microfluidic devices.

Acknowledgements

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