

# High-throughput assay for screening the stability of peptide drugs in proteolytic environments

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## The Challenge

Peptides or peptidomimetics are up-and-coming drug candidates because they promise to combine the best of both worlds: the ease of synthesis of small molecules and the excellent recognition properties biomolecules usually display. Yet, the notorious susceptibility of peptide drugs to proteolytic degradation has limited their use to parenteral administration. In order to identify candidate peptides suitable for oral delivery assays are needed with which the stability of peptides towards digestive juices can be determined. Currently available methods have significant drawbacks including impaired accessibility of the peptidic substrates, need for substrate-specific antibodies or constraints in peptide length and properties. Thus, novel assays are required that lack these disadvantages but at the same time are sensitive, easy-to-handle and high-throughput capable.

Assay based on	Assay characteristics				
	Enzyme kinetics determinable	High-throughput compatible	Operative with high variety of peptides	High intrinsic robustness	
State of the art assays	Physical Separation	+	-	-	-
	Solid phase bound peptides	-	+	+	+
	Chromogenic / FRET-substrates	+	+/-	-	+/-
Novel Assay	Detection of double-tagged peptides	+	+	+	+

**Advantages of the novel high-throughput peptide stability assay.** Source: Bade, Röckendorf and Frey 2006.

## The Technology

The novel assay is able to rapidly and accurately determine the susceptibility of peptides or peptidomimetics towards raw enzyme extracts or purified enzymes and is well suited for the parallel analysis of many different peptide sequences. Substrate specific antibodies are not required as the peptides are equipped on both ends with different labels, for example biotin and a 2,4-dichlorophenoxyacetic acid derivative. The double-labeled peptides are synthesized in parallel on a solid support and are subsequently cleaved-off. Hence, the proteolysis reaction can be performed in solution ensuring excellent accessibility of potential cleavage sites. The assay is designed to detect only intact peptides down to 100 amole amounts and thus records the loss of peptide upon protease exposure. Due to the assay design >95% of all peptides can be analyzed at first go and cleavage follows pseudo-first order kinetics which allows the calculation of *in vivo* peptide half-lives when the dilution of the enzyme is known.

## Commercial Opportunity

Licensing

## Patent Situation

A priority establishing German patent application was filed in 2005. A PCT-application was filed in 2006.

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