REGENERABLE SURFACE PLASMON RESONANCE (SPR) SURFACES FOR RELIABLE, COST-EFFECTIVE, AND WIDE-RANGING APPLICATIONS

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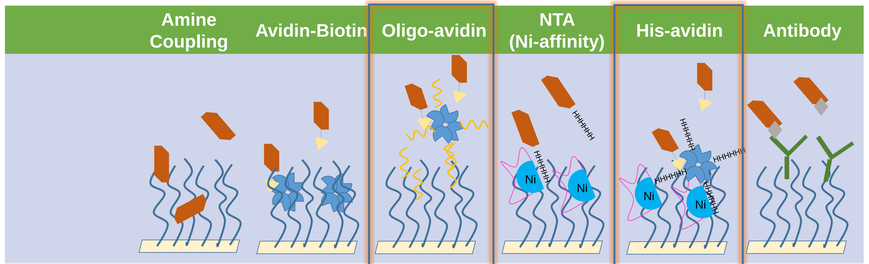
COMM04

Abstract

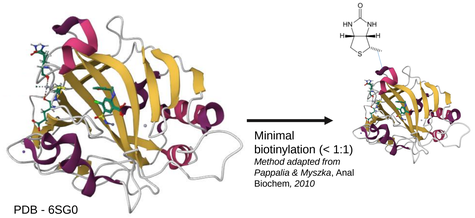
Traditional SPR-based drug discovery efforts in the small-molecule space have revolved around single-use chips for screening and hit-to-lead efforts. Typically, proteins are recombinantly or chemically biotinylated for capture on custom-made or, more recently, pre-coated (strept)avidin chips. These surfaces are unable to be regenerated for reuse with freshly immobilized target protein due to the sub-picomolar affinity of the biotin-avidin interaction and the durability of the capture-protein to harsh chemical exposure. Inspired by recent technological advances, we have established simple regenerable protocols that can (1) accommodate target-proteins with common tags such as His- and Avi-, (2) reach sufficient surface density for small-molecule testing, and (3) maintain a stable-baseline for accurate kinetic measurements. On a Biacore 8k+ system, these protocols make effective use of chips, saving time and reducing waste. Additionally, the simple replenishment process allows for special applications such as testing challenging targets with poor longevity on the surface, or quasi-stable multi-component complexes. Through regeneration, we can efficiently test small covalent ligands, a process that was previously considered low-throughput and expensive. The methods are broadly applicable for both early-stage screening and late-stage characterizations by measuring affinities of candidates with long residence times, leveraging residual occupancy (aka chaser) formats.

Comparison of key SPR-based capture strategies



	Amine Coupling	Avidin-Biotin	Oligo-avidin	NTA (Ni-affinity)	His-avidin	Antibody
Chip Type	CM5	SA / NA	CAP / RGD	NTA	NTA	CM5 / PAG
Regenerable	X	X	✓	✓	✓	✓
Density	High	High	Low/medium	High	Medium	Medium/low
Stability	+++	+++	+++	+/++	++/+++	++
Oriented	No	Yes	Yes	Yes	Yes	Yes
PTS	Moderate	High	Moderate	Moderate	High	Moderate

Model System for Small-molecules: Carbonic Anhydrase



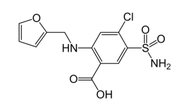
PDB - 6SG0

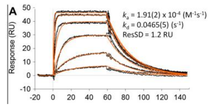
CA II complexed with furosemide

Minimal biotinylation (< 1:1)

Method adapted from Pappalia & Myszka, Anal Biochem, *2010*

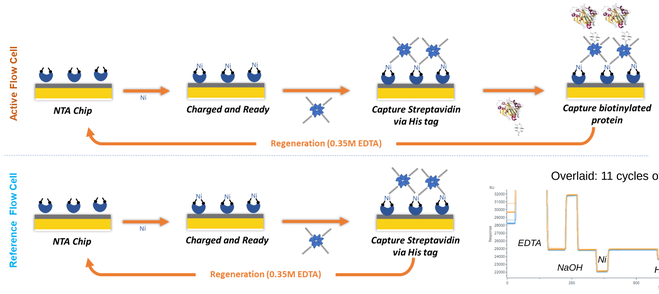
Well-studied control: furosemide



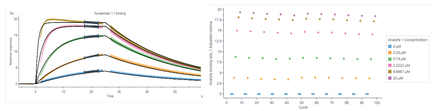


From Quinn J, Anal Biochem *2012*

Strategy 1: His-Streptavidin (NTA Chip)

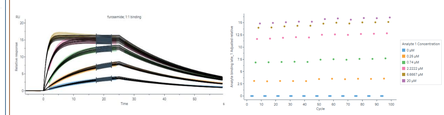


No regeneration



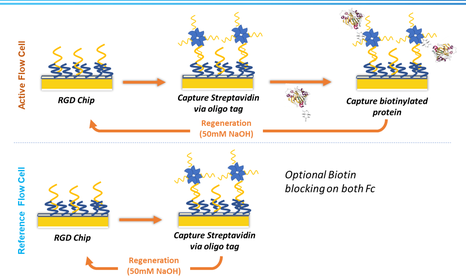
Sample	K$_D$ (nM)	k$_a$ (M-1s-1)	k$_d$ (s-1)	Rmax
furosemide 1	531	65900	0.035	19.7
furosemide 2	540	64600	0.0349	19.7
furosemide 3	525	64800	0.034	19.5
furosemide 4	557	63000	0.0351	19.5
furosemide 5	536	64600	0.0346	19.3
furosemide 6	554	63400	0.0351	19.4
furosemide 7	556	63500	0.0353	19.3
furosemide 8	572	62200	0.0356	19.2
furosemide 9	562	63000	0.0354	19
furosemide 10	572	62800	0.0359	18.9
furosemide 11	568	62500	0.0355	18.7
AVERAGE	**552**	**63664**	**0.035**	**19.3**
std dev	16	1098	0.0005	0.3
CV	2.9%	1.7%	1.4%	1.6%

With regeneration

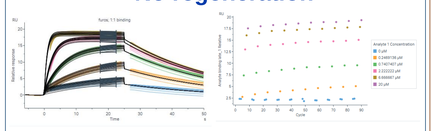


Sample	K$_D$ (nM)	k$_a$ (M-1s-1)	k$_d$ (s-1)	Rmax
furosemide 1	494	71000	0.035	15.2
furosemide 2	485	71000	0.034	15.4
furosemide 3	484	70900	0.034	15.5
furosemide 4	484	70300	0.034	15.7
furosemide 5	479	70800	0.034	15.7
furosemide 6	486	69900	0.034	15.8
furosemide 7	493	69800	0.034	16
furosemide 8	484	70600	0.034	15.9
furosemide 9	478	71300	0.034	16.1
furosemide 10	484	71300	0.035	16.2
furosemide 11	481	71300	0.034	16.2
AVERAGE	**485**	**70745**	**0.034**	**15.8**
std dev	5	514	0.0003	0.3
CV	1.0%	0.7%	0.9%	2.0%

Strategy 2: Oligo-Streptavidin (XanTec RGD chip)

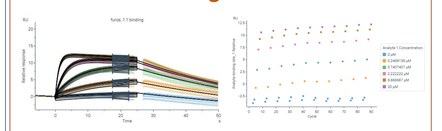


No regeneration



Sample	K$_D$ (nM)	k$_a$ (M-1s-1)	k$_d$ (s-1)	Rmax
furosemide 1	805	48300	0.039	17.6
furosemide 2	697	51500	0.036	17.8
furosemide 3	646	53900	0.035	17.9
furosemide 4	618	55300	0.034	17.8
furosemide 5	589	56500	0.033	17.8
furosemide 6	563	58800	0.033	17.8
furosemide 7	569	68500	0.039	18.2
furosemide 8	551	71500	0.039	18.1
furosemide 9	549	72500	0.040	18.1
furosemide 10	547	68400	0.037	18
AVERAGE	**614**	**60520**	**0.037**	**17.9**
std dev	79	8431	0.002	0.2
CV	13%	14%	7%	1%

With regeneration

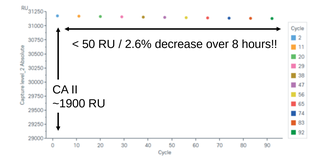


Sample	K$_D$ (nM)	k$_a$ (M-1s-1)	k$_d$ (s-1)	Rmax
furosemide 1	659	40800	0.027	11.8
furosemide 2	642	41300	0.027	11.9
furosemide 3	587	44600	0.026	12.1
furosemide 4	544	46100	0.025	12.3
furosemide 5	497	50100	0.025	12.4
furosemide 6	489	48900	0.024	12.5
furosemide 7	477	48800	0.023	12.6
furosemide 8	467	50300	0.024	12.6
furosemide 9	452	51600	0.023	12.7
furosemide 10	431	53600	0.023	12.7
AVERAGE	**525**	**47610**	**0.025**	**12.4**
std dev	76	4072	0.001	0.3
CV	15%	9%	6%	3%

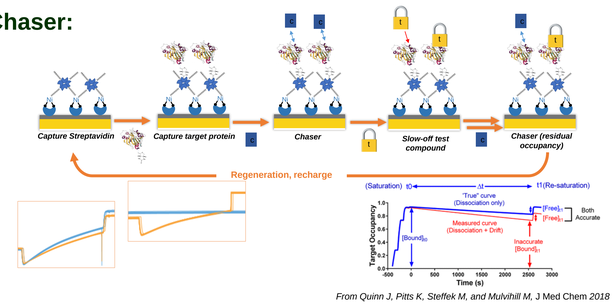
Advanced Applications: Chaser, Ternary complex

Highly stable and regenerable surface opens opportunities to measure **long off-rates.**

Sufficient stability observed on His-Streptavidin surface:

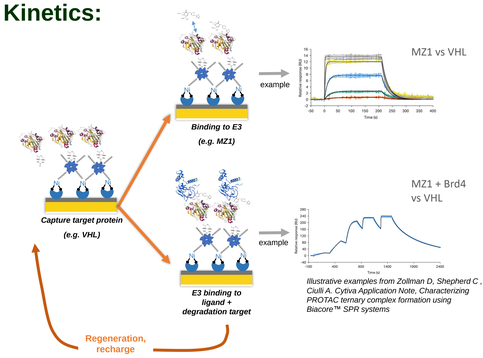


Chaser:



From Quinn J, Pitts K, Steffek M, and Mulvihill M, J Med Chem *2018*

Ternary Kinetics:



Illustrative examples from Zollman D, Shepherd C , Ciulli A. Cytiva Application Note, Characterizing PROTAC ternary complex formation using Biacore™ SPR systems

Summary

- **2 highly effective capture strategies have been implemented at WuXi AppTec to perform sophisticated SPR analysis of small molecules using reproducible, reusable chip surfaces**
- **His-Streptavidin makes highly stable complexes, allowing for direct testing of small molecules**
  - **Can regenerate to provide high-quality kinetic measurements or apply multiple walk-away cycles of advanced formats such as the chaser**
- **A functionally similar oligo-streptavidin surface can accomplish the same tasks, though with slightly lower protein densities**

References

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- Quinn J, Pitts K, Steffek M, and Mulvihill M, Determination of Affinity and Residence Time of Potent Drug-Target Complexes by Label-free Biosensing**,** *J Med Chem* 2018 61 (12), 5154-5161

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