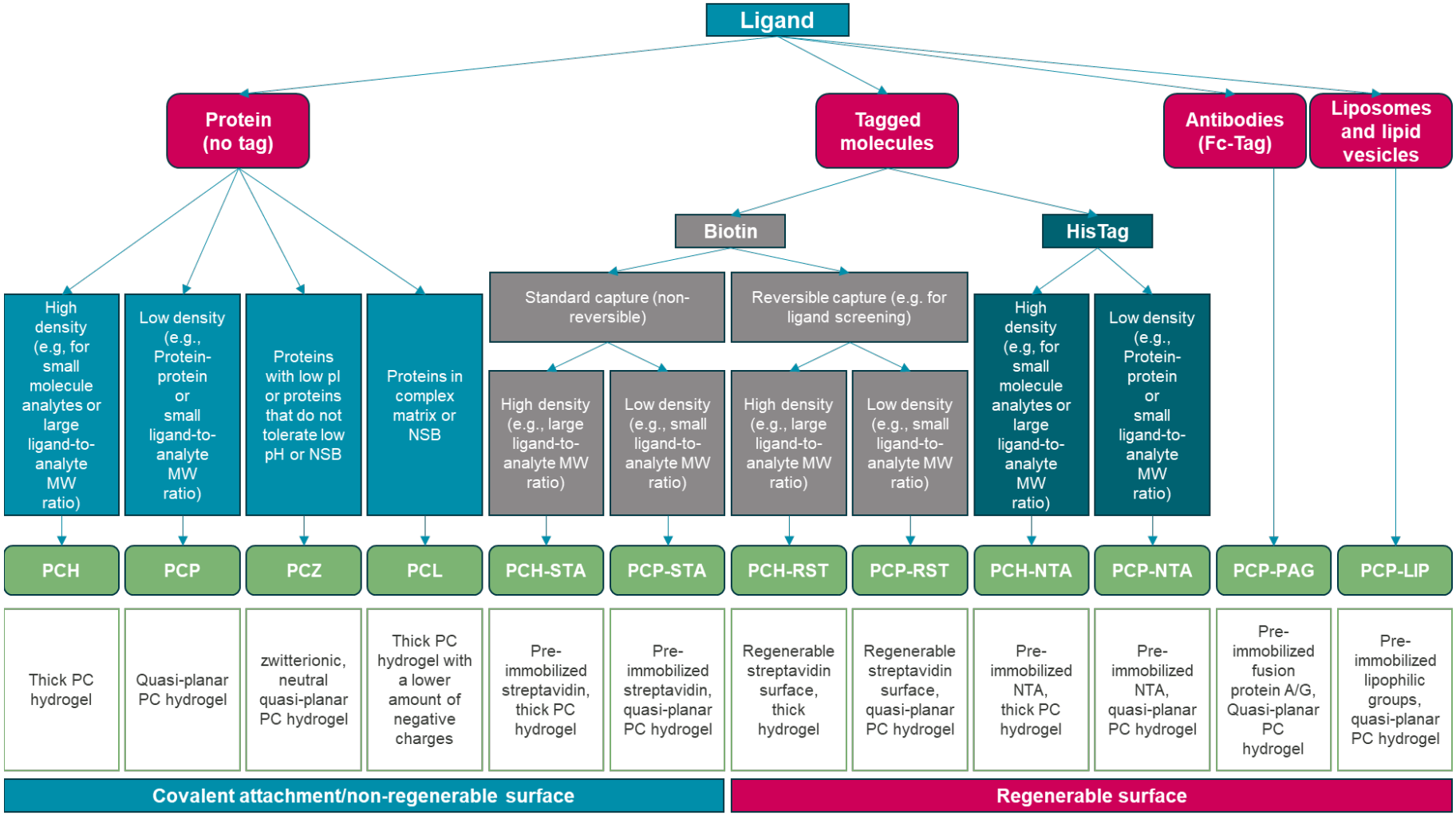
Malvern Panalytical

KYMAchip Protocols

Get to know them all

Index

KYMAchip decision tree



Amine-coupling of molecules to PCP, PCH, and PCL KYMAchips

Overview

This protocol gives recommendations for the immobilization of molecules (ligands) to PCP, PCH, and PCL KYMAchips via amine coupling. Amine coupling is a common and reliable method to covalently immobilize protein ligands for surface-based kinetics measurement methods. Immobilization occurs via free primary amine groups such as those on lysine residues, which are abundant in most proteins, or at the N-terminus of proteins and peptides.

The linear polycarboxylate matrix on the KYMAchip surface is first activated with a mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to create reactive succinimide esters. The ligand is subsequently injected in a low salt buffer lacking primary amines at a pH below the isoelectric point (pI) of the ligand. Under these conditions, the ligand becomes positively charged and is efficiently attracted (preconcentrated) to the negatively charged matrix. A high local concentration of ligand maximizes the efficiency of amine coupling. After ligand immobilization, the surface is passivated using an ethanolamine solution to quench unreacted esters. For more information refer to the *Kinetics Guide Section 3.1.3.1*.

PCH and PCP are both composed of polycarboxylates with the same charge density. PCH has a thicker matrix than PCP, giving it a higher ligand capacity. PCL has a similar thickness to PCH but contains fewer carboxyl groups. As PCL carries fewer negative charges, its binding capacity is similar to PCP. This reduced charge density is designed to help minimize non-specific binding.

Chip types:

PCP: thin, lower ligand capacity, high negative charge
PCH: thick, higher ligand capacity, high negative charge
PCL: thick, lower ligand capacity, low negative charge

Amine coupling creates a permanent covalent attachment between the ligand and the chip surface, preventing removal during regeneration. In conjunction with the WAVEsystem, PCH, PCP, and PCL KYMAchips provide a convenient method for kinetic measurements of untagged ligands with various analytes.

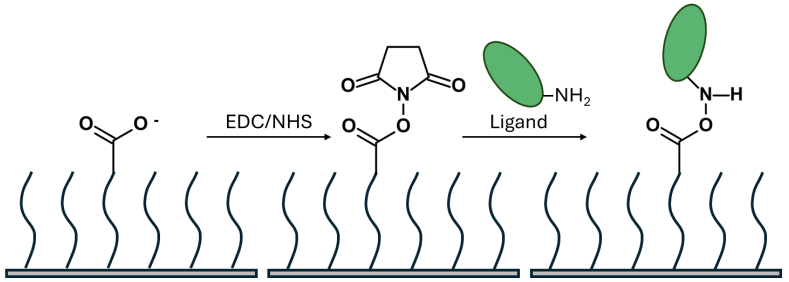


Figure 1. Schematic representation of PCH/PCP/PCL KYMAchip surface. The surface is first activated with a mixture of EDC and NHS, after which the ligand (green) is immobilized.

Materials required

- PCP, PCH or PCL KYMAchip
- Borate buffer (0.1 M borate, pH 9, 1 M NaCl)
- 0.2 x running buffer
- Activation reagents
  - 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)
  - 100 mM N-hydroxysuccinimide (NHS) or N-hydroxysulfosuccinimide (sulfo-NHS) in 50 mM MES pH 5.0
- Passivation reagent (1 M ethanolamine, pH 8)
- Ligand in coupling buffer. For a protein solution a concentration of 10-50 µg/mL is typical.
- Recommended coupling buffers:
  - 2-20 mM sodium formate, pH 3.0 - 4.0
  - 2-20 mM sodium acetate, pH 4.0 - 5.5
  - 1-10 mM sodium maleate, pH 5.5 - 7.0

  The coupling buffer pH should be 0.5-1 pH unit below the pI of the ligand.

Procedure

Conditioning

Condition a KYMAchip by using the conditioning wizard in WAVEcontrol software. Select PCP, PCH, or PCL as KYMAchip type. Standard conditioning cycles will be generated (Table 1).

Table 1. Standard conditioning cycles.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Conditioning	Borate buffer	10	180	60
2	Startup	0.2 x running buffer	10	60	60
3	Startup	0.2 x running buffer	10	60	60
4	Startup	0.2 x running buffer	10	60	60

Immobilization

Use the immobilization wizard for ligand coupling. Select "Amine coupling to carboxymethyl surface". Select channel(s) (flow paths) to be activated and passivated (active and reference channels) and add information about the ligand(s) to be immobilized on active channels. A series of cycles will be created (Table 2).

Table 2. Standard cycles for amine coupling. Parameters may be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Activation	EDC/NHS	10	420	60
2	Immobilization	Ligand	10	420	60
3	Passivation	1 M ethanolamine pH 8	10	420	60

Notes for immobilization

- 0.2x running buffer is recommended during an immobilization series. Tris-based buffers or buffers with primary amines are not compatible with amine coupling.
- Reference channel activation and passivation are required for appropriate referencing and data evaluation.
- Activation with EDC/NHS is done to activate carboxylic groups for amine coupling. Use 100 mM EDC (in water) and 100 mM NHS (in 50 mM MES buffer, pH 5). Mix 1:1 (v/v) just before starting the series. The standard activation time recommended for KYMAchips is 7 minutes but can be adjusted accordingly.
- Immobilization is done by injecting the ligand solution over the activated surface. Default contact time is 420 s but can be adjusted accordingly. Dilute the ligand in coupling buffer (low salt concentration and a pH around 0.5-1 unit below the pI) for successful preconcentration and immobilization. Ligand concentrations between 10 and 50 µg/mL (or even between 5 and 100 µg/mL depending on the ligand) are typical for immobilization.
- For initial binding assessment, we recommend using immobilization levels that yield a theoretical R_{max} between 20 and 100 pg/mm^2. For optimized data quality, consider lowering the immobilization level to reach an R_{max} between 5 and 50 pg/mm^2.

- Passivation of the remaining active groups is done by injection of 1 M ethanolamine, pH 8, for 420 s.

Kinetics

- Switch to a physiological buffer of your choice and perform a buffer exchange to remove the previous buffer from the flow paths. Begin the interaction measurements.
- Use one of the available wizards to run kinetics (e.g. RAPID Kinetics or Kinetics). The settings of the kinetics experiment should be based on the expected affinity of the interaction.
- It is recommended to run a dilution series of at least five concentrations of the analyte (0.1 x K_D to 10 x K_D) for traditional multicycle kinetics, or one concentration (10 x K_D or higher) for waveRAPID series. For more information refer to the *Kinetics Guide Section 3.5*.

Tips for optimal performance

- Always prepare the EDC/NHS mixture immediately before starting the immobilization series.
- Quality of immobilization reagents is crucial for amine coupling to be successful. Use only high-quality reagents dedicated to ligand immobilization.
- Note that the half-life of the reactive succinimide ester is in the order of minutes at pH 8-9 up to a few hours at pH 4-5 in aqueous buffers.
- The coupling buffer can be optimized before the immobilization step using pH scouting wizard, by injecting the ligand in different buffers at different pH over the nonactivated surface (*Kinetics Guide Section 3.1 – pH scouting*). An increase in signal over time shows the pre-concentration of the ligand. At the end of the injection the ligand is washed out again. When choosing a buffer, the protein stability in that buffer should be also considered. The highest pH giving good preconcentration should be selected.
- Proteins can exhibit limited stability when exposed to low-ionic-strength, low-pH solutions used for preconcentration. To minimize protein exposure to undesirable conditions, dilute the ligand in coupling buffer prior to use.
- Amine coupling immobilizes proteins in a random orientation, rendering some binding sites inaccessible to the analyte. Take this into consideration when calculating ligand density.

Troubleshooting

If ligand immobilization levels are low, check the measurements and identify the cause of low ligand levels.

If preconcentration is not satisfactory (little increase of response during ligand injection):

- Check preconcentration conditions using pH scouting.
- Make sure that the ionic strength of the ligand buffer is low (ligand is sufficiently diluted or desalted).
- Increase the concentration of the ligand and/or contact time.
- Check the shape of EDC/NHS injection. If the signal increases significantly during the second part of the injection, consider reducing the EDC concentration or switching to an alternative EDC.

If the ligand is preconcentrated on the surface (high response during injection) but not immobilized (low level in baseline after injection):

- Make sure to use fresh activation reagents.
- Ensure that buffers do not contain primary amines (e.g., Tris) or sodium azide.
- Consider different immobilization/capture method.

If the immobilization level seems sufficient, but analyte responses are too low:

- Increase the immobilization level of the ligand on the surface. Amine-coupled ligands typically exhibit lower binding activity than captured ligands.
- Check the stability of protein in the low pH coupling buffer.
- Consider different immobilization/capture methods.

Regeneration

When working with tight-binding interactions, regeneration of the KYMAchip surface is necessary. Since amine-coupled ligands are irreversibly bound to the surface only the analyte-ligand interaction can be disrupted. It is recommended to perform scouting experiments to find optimal regeneration conditions. Common procedures for regeneration of the ligand surface consist of short injection pulses of solutions with extreme pH levels, high salt concentration, or interaction-specific reagents (Table 3).

Table 3. Regeneration solutions.

Interaction type	Regeneration solution	Injection duration
Protein (e.g. anti-IgG or protein A)	10 mM Glycine pH 1.5-2.5 or 100 mM H_3PO_4	One or more injections of 30 s
ssDNA-ssDNA	6-7 M Urea or 100 mM NaOH	30 s injection *
Hydrophobic	0.2 M CHAPS or other detergent	3 min injection
Charge driven	0.5-1 M NaCl or high/ low pH buffers	30 s injection *
Displacement (small molecule – enzyme)	High conc. competitor (low affinity)	30 s - 3 min injection *

** Phase jumps can occur during regeneration*

We suggest keeping the regeneration conditions as mild as possible to not damage the ligand. To reduce exposure of the ligand to the regeneration solution, the solutions should be injected for a short duration, but this process can be repeated to ensure full regeneration of the ligand. For more information refer to the *Kinetics Guide Section 3.1.4*.

Additional information

For more details, please visit our *Knowledge Center* (https://www.malvernpanalytical.com/en/learn/knowledge-center) or consult the *Kinetics Guide – Binding Kinetics with the WAVEsystem*.

The guide can also be found in WAVEcontrol under the Help tab.

Table 4: Ordering information

Consumable	Product number
KYMAchip PCP	10081626
KYMAchip PCH	10081623
KYMAchip PCL	10081592
KYMAchip DXP *	10081625
KYMAchip DXH *	10081593

** DXP and DXH chips are available upon request. These DX chips utilize a carboxymethyl dextran matrix and follow the same protocol as polycarboxylate surfaces. However, for GCI, the recommended primary surfaces are PC chips, which feature a polycarboxylate matrix.*

Capture of His-tagged molecules to PCP- and PCH-NTA KYMAchips

Overview

This protocol gives recommendations for capturing polyhistidine-tagged molecules (ligands) to PCP- and PCH-NTA KYMAchips via nickel (Ni^{2+})/nitrilotriacetic acid (NTA). NTA is a chelating agent that forms coordination compounds with metal ions (such as Ni^{2+}). The resulting complex interacts with histidine molecules due to the intrinsic ability of the imidazole groups to chelate nickel (II) nitriloacetate. His-tag capturing enables site-directed, homogenous immobilization of His-tagged ligands in physiological conditions without significant changes of ionic strength or pH.

The NTA KYMAchip surface can be regenerated using chelators such as EDTA or imidazole to remove previously captured ligands. Alternatively, ligand capture can be stabilized by His-tag-guided amine coupling, resulting in covalent immobilization of the ligand on the chip surface.

In conjunction with the WAVEsystem, NTA KYMAchips provide a convenient method for performing kinetic measurements of His-tagged ligands with various analytes.

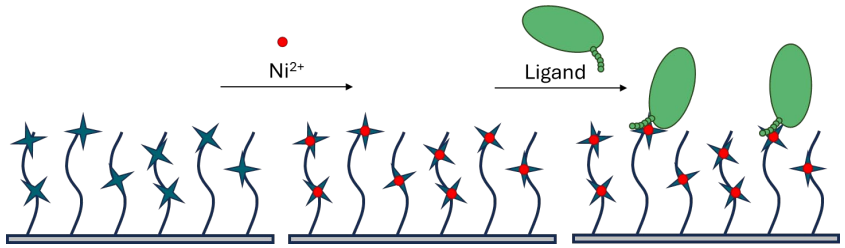


Figure 2. Schematic representation of NTA KYMAchip surface. Polycarboxylate matrix is pre-immobilized with NTA (blue). The surface is first activated with nickel ions (Ni^{2+}, red), then His-tagged ligand (green) is captured. The surface is ready for molecular interactions analysis.

Materials required

- PCP-NTA or PCH-NTA KYMAchip
- Borate buffer (0.1 M borate, pH 9, 1 M NaCl)
- 0.25 M and 0.35 M EDTA
- Running buffer
- His-tagged ligand
- Activation reagent (0.5 mM $NiCl_2$)

Optional amine coupling reagents:

- 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)
- 100 mM N-hydroxysuccinimide (NHS) in 50 mM MES pH 5.0
- 1 M ethanolamine, pH 8

Procedure: His-tag capture

Conditioning

Condition a KYMAchip by using the conditioning wizard in WAVEcontrol software. Select PCP-NTA or PCH-NTA. Standard conditioning cycles will be generated (*Table* 5).

Table 5. Standard conditioning cycles.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Conditioning	Borate buffer	10	180	60
2	Conditioning	0.25 M EDTA	10	180	60
3	Startup	Running buffer	10	60	60
4	Startup	Running buffer	10	60	60
5	Startup	Running buffer	10	60	60

Capture

Use the immobilization wizard to capture ligands. Select "Capturing of His-tagged ligands to Ni-NTA surface". Select channel(s) (flow paths) to be activated and add information about the ligand. A series of cycles will be created (Table 6).

Table 6. Standard cycles for His-tag capture. Parameters can be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Startup	Running buffer	10	60	60
2	Startup	Running buffer	10	60	60
3	Activation	0.5 mM $NiCl_2$	10	120	60
4	Startup	Running buffer	10	60	60
5	Capture	His-tagged ligand	10	420	60
6	Startup	Running buffer	10	60	60

Notes for capture

- Including 50 µM EDTA in the buffer is recommended to reduce interference from trace metal contaminants. Avoid using imidazole, other chelating agents, and bivalent metal ions (e.g., Ca^{2+}, Zn^{2+}, Cu^{2+}) in the buffer, as they can disrupt Ni-NTA binding.
- During capture cycles, avoid phosphate running buffers, as this can cause nickel ions to precipitate.
- Startup cycles equilibrate the surface and allow the response to stabilize before the next cycle.
- Activation of NTA is done by injecting 0.5 mM $NiCl_2$ in running buffer or water for 120 s. Avoid activating the reference channel to reduce risk of non-specific binding. If using a reference His-tagged protein, activate and capture it the same way as the ligand.
- Capture is done by injecting a solution containing the ligand over the activated surface. Default contact time is 420 s but this can be adjusted accordingly. The ligand should be prepared in running buffer, with typical concentrations between 10 and 50 µg/mL.
- For initial binding assessment, we recommend using immobilization levels that yield a theoretical R_{max} between 20 and 100 pg/mm^2. For optimized data quality, consider lowering the level to reach an R_{max} between 5 and 50 pg/mm^2.

Kinetics

- If a different running buffer is required for kinetic measurements, perform a buffer exchange to remove the previous buffer from the flow paths.
- Use one of the available wizards to run kinetics (e.g. RAPID Kinetics or Kinetics). The settings of the kinetics experiment should be based on the expected affinity of the interaction.
- It is recommended to run a dilution series of at least five concentrations of the analyte (0.1 x K_D to 10 x K_D) for traditional multicycle kinetics or one concentration (10 x K_D or higher) for a waveRAPID series. For more information refer to the *Kinetics Guide Section 3.5*.

Tips for optimal performance & troubleshooting

If the response after ligand capture is unstable and the signal drifts:

- Include multiple startup cycles before the kinetic assay to stabilize the surface.
- Reduce the amount of captured ligand.
- Consider His-tag-guided amine coupling to prevent ligand dissociation; but avoid using it with crude samples such as cell lysates due to potential co-binding of non-target proteins.
- Use ligands with extended His-tags (e.g., 10x or 12xHis) for improved capture stability. The stability of the capture depends on both the nature of the protein and the accessibility of the His-tag.
- For detergent-solubilized ligands, it is recommended to perform 30-50 startup cycles before initiating the kinetic series to stabilize the sensor surface.

Regeneration

- Regeneration can be used during ligand screening or when working with tight binders. It's typically integrated into the assay workflow and configured via software wizards.
- Inject 0.35 M EDTA (or other chelators, such as imidazole) for 60 seconds, followed by a 60-second rinse. If needed, repeat the cycle.
- Note that regeneration removes both nickel ions and His-tagged molecules from the surface. Before the next capture step, re-load the surface with nickel ions using 0.5 mM $NiCl_2$.

Procedure: His-tag-guided amine coupling

Conditioning

Condition a KYMAchip by using the conditioning wizard in WAVEcontrol software. Select PCP-NTA or PCH-NTA. Standard conditioning cycles will be generated (*Table 7*).

Table 7. Standard conditioning cycles.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Conditioning	Borate buffer	10	180	60
2	Conditioning	0.25 M EDTA	10	180	60
3	Startup	Running buffer	10	60	60
4	Startup	Running buffer	10	60	60
5	Startup	Running buffer	10	60	60

Immobilization

To immobilize the ligand by His-tag-guided amine coupling use the immobilization wizard and select "Capture and amine coupling of His-tagged ligands to Ni-NTA surface". Select the channel(s) to be activated and add information about the ligand. Nickel, EDTA, and Startup cycles are added by default. A series of cycles will be created (

Table 8).

Table 8. Standard cycles for His-tag-guided amine coupling. Parameters can be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Activation	0.5 mM $NiCl_2$	10	120	60
2	Startup	Running buffer	10	60	60
3	Startup	Running buffer	10	60	60
4	Activation	EDC/NHS	10	420	60
5	Capture	His-tagged ligand	10	420	60
6	Passivation	Ethanolamine	10	420	60
7	Startup	Running buffer	10	60	60
8	Conditioning	0.35 M EDTA	10	420	60

Notes for capture

- Including 50 µM EDTA in the buffer is recommended to reduce interference from trace metal contaminants. Avoid using imidazole, other chelating agents, and bivalent metal ions (e.g., Ca^{2+}, Zn^{2+}, Cu^{2+}) during capture step, as they can disrupt Ni-NTA binding.
- During capture cycles, avoid phosphate running buffers, as this can cause nickel ions to precipitate.
- Activate the NTA surface with nickel ions using 0.5 mM $NiCl_2$ in running buffer or water. The standard activation time is 120 s. The empty reference channel should be activated and passivated the same way as the ligand channel (remaining nickel ions are removed by EDTA in the last step). When using a negative control protein

on the reference channel, immobilize the control His-tagged protein the same way as ligand.

- Activation with EDC/NHS is done to activate carboxylic groups for amine coupling. Use 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (in water) and 100 mM N-hydroxysuccinimide (NHS) (in 50 mM MES buffer, pH 5). Mix 1:1 (v/v) just before before starting the series.
- Capture coupling is done by injecting a solution containing the ligand over the activated surface. Default contact time is 420 s but this can be adjusted accordingly. Ligand should be prepared in running buffer, with typical concentrations between 10 and 50 µg/mL.
- Since initial attachment of the ligand is driven by His-tag capture and not by electrostatic preconcentration, physiological buffers can be used for immobilization (unlike standard amine coupling procedures for PCH/PCP/PCL chips).
- For initial binding assessment, we recommend using ligand levels that yield a theoretical R_{max} between 20 and 100 pg/mm^2. For optimized data quality, consider lowering the immobilization level to reach an R_{max} between 5 and 50 pg/mm^2.
- Deactivation of the remaining active amine groups is done by injecting 1 M ethanolamine, pH 8 for 420 s.
- Nickel ions and the ligand molecules that were not coupled to the surface are removed by injecting 0.35 M EDTA for 420 s.

Kinetics

- If a different running buffer is required for kinetic measurements, perform a buffer exchange to remove the previous buffer from the flow paths.
- Use one of the available wizards to run kinetics (e.g. RAPID Kinetics or Kinetics). The settings of the kinetics experiment should be based on the expected affinity of the interaction.
- It is recommended to run a dilution series of at least five concentrations of the analyte (0.1 x K_D to 10 x K_D) for traditional multicycle kinetics or one concentration (10 x K_D or higher) for a waveRAPID series.

Tips for optimal performance & troubleshooting

- Always prepare EDC/NHS mixture just before starting the immobilization series.

If the response after ligand capture is unstable:

- Include multiple startup cycles before the kinetic assay to stabilize the surface.
- For detergent solubilized ligands, we recommend adding 30-50 startups before starting the series to stabilize the surface.
- His-tag-guided amine coupling is not recommended for crude matrices such as cell lysates. Activated groups on the surface may covalently link both His-tagged and non-His-tagged proteins to the surface.

Regeneration

- The surface cannot be regenerated when using His-tagged guided amine coupling, but ligand regeneration (removal of analyte from the ligand, e.g., when working with tight binders) is still possible. Regeneration parameters are project dependent. For more information refer to the *Kinetics Guide Section 3.1.4*.

Additional information

For more details, please visit our *Knowledge Center* (https://www.malvernpanalytical.com/en/learn/knowledge-center) or consult the *Kinetics Guide – Binding Kinetics with the WAVEsystem*.

The guide can be also found in WAVEcontrol under Help tab.

Table 9: Ordering information

Consumable	**Product number**
KYMAchip PCP-NTA	10081628
KYMAchip PCH-NTA	10081624

Capture of biotinylated molecules to PCP- and PCH-STA KYMAchips

Overview

This protocol gives recommendations for capturing biotinylated molecules (ligands), such as proteins, peptides and nucleic acids, to PCP- and PCH-STA KYMAchips via the biotin-streptavidin (STA) interaction. Biotin-streptavidin is one of the tightest non-covalent interactions found in nature, with a reported K_D of 10^{-15} M. The linear polycarboxylate matrix on the KYMAchip surface is functionalized with covalently bound streptavidin. This allows the capture of biotinylated ligands in physiological conditions without significant changes of ionic strength or pH of the buffer.

The biotinylated ligand binds irreversibly to the STA sensor surface, which means that the captured ligands cannot be removed in the regeneration step.

In conjunction with the WAVEsystem, STA KYMAchips provide a convenient method for kinetic measurements of biotinylated ligands with various analytes.

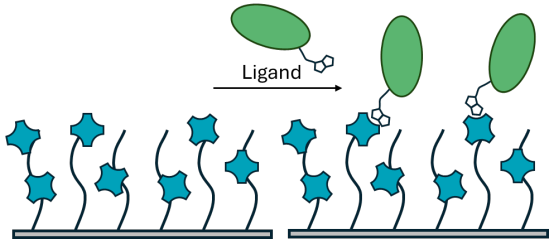


Figure 3. Schematic representation of STA KYMAchip surface. Polycarboxylate matrix is pre-immobilized with streptavidin (blue). The biotinylated ligand (green) gets captured by streptavidin. The surface is then ready for molecular interaction analysis.

Materials required

- PCP-STA or PCH-STA KYMAchip
- Borate buffer (0.1 M borate, pH 9, 1 M NaCl)
- Running buffer
- Biotinylated ligand

Notes

- Desorb the instrument before starting the experiment with streptavidin KYMAchips.
- Make sure to let the chip equilibrate to room temperature before opening.

Procedure

Conditioning

Condition a KYMAchip by using the conditioning wizard in WAVEcontrol software. Select PCP-STA or PCH-STA as KYMAchip type. Standard conditioning cycles will be generated (*Table* 10).

Table 10. Standard conditioning cycles.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Conditioning	Borate buffer	10	180	60
2	Startup	Running buffer	10	60	60
3	Startup	Running buffer	10	60	60
4	Startup	Running buffer	10	60	60

Capture

Use the immobilization wizard for the ligand capture step. Select "Capturing biotinylated ligand to streptavidin surface" and add information about the ligand. A series of cycles will be created (*Table* 11).

Table 11. Standard cycles for capture on STA KYMAchips. Parameters can be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Startup	Running buffer	10	60	60
2	Startup	Running buffer	10	60	60
3	Capture	Ligand	10	420	60
4	Startup	Running buffer	10	60	60

Notes for Capture

- Startup cycles equilibrate the surface and allow the response to stabilize before the next cycle.
- Capture is done by injecting the ligand solution over the sensor surface. Default contact time is 420 s but can be adjusted accordingly.
- For initial binding assessment, we recommend using capture levels that yield a theoretical R_{max} between 20 and 100 pg/mm^2. For optimized data quality, consider lowering the immobilization level to reach an R_{max} between 5 and 50 pg/mm^2.
- Ligand capture level can be controlled by using the Target Level function.
- Ligand should be prepared in the running buffer. Typical protein ligand concentrations are between 5 and 100 µg/mL. For nucleic acid ligands, concentrations between 50 nM and 1 µM are recommended.

Kinetics

- Use one of the available wizards to run kinetics (e.g. RAPID Kinetics or Kinetics). The settings of the kinetics experiment should be based on the expected affinity of the interaction.
- It is recommended to run a dilution series of at least five concentrations of the analyte (0.1 x K_D to 10 x K_D) for traditional multicycle kinetics, or one concentration (10 x K_D or higher) for waveRAPID series. For more information refer to the *Kinetics Guide Section 3.5*.

Tips for optimal performance & troubleshooting

If the ligand levels are low:

- Increase injection time, number of capture cycles, or ligand concentration.
- Consider construct optimization for recombinant proteins or different capture/immobilization methods.

If the response after capture is not stable:

- Include several startup cycles before the kinetic assay to help stabilize the surface.
- Consider reducing the amount of captured ligand to improve stability.
- For detergent-solubilized ligands, we recommend performing between 30 and 50 startup cycles prior to the kinetic series to ensure a stable baseline.

Regeneration

When working with tight-binding interactions, regeneration of the KYMAchip surface is necessary. Since biotinylated ligands are very tightly bound to the streptavidin on the surface only the analyte-ligand interaction can be disrupted. It is recommended to perform scouting experiments to find optimal regeneration conditions. Common procedures for regeneration of the ligand surface consist of short injection pulses of solutions with extreme pH levels, high salt concentration, or interaction-specific reagents (*Table 12*).

If there is no suitable regeneration method available for the studied interaction, consider using regenerable streptavidin KYMAchips (PCP/PCH-RST). In this case, the surface can be regenerated by removing the streptavidin with the ligand-analyte complex attached. For further details, please refer to the PCP/PCH-RST protocol.

Table 12. Regeneration solutions.

Interaction type	Regeneration solution	Injection duration
Protein (e.g. anti-IgG or protein A)	10 mM Glycine pH 1.5-2.5 or 100 mM H_3PO_4	One or more injections of 30 s
ssDNA-ssDNA	6-7 M Urea or 100 mM NaOH	30 s injection *
Hydrophobic	0.2 M CHAPS or other detergent	3 min injection
Charge driven	0.5-1 M NaCl or high/ low pH buffers	30 s injection *
Displacement (small molecule – enzyme)	High conc. competitor (low affinity)	30 s-3 min injection *

** Phase jumps can occur during regeneration*

Additional information

For more details, please visit our *Knowledge Center* (https://www.malvernpanalytical.com/en/learn/knowledge-center) or consult the *Kinetics Guide – Binding Kinetics with the WAVEsystem*.

The guide can also be found in WAVEcontrol under the Help tab.

Table 13: Ordering information

Consumable	Product number
KYMAchip PCP-STA	10081596
KYMAchip PCH-STA	10081621
KYMAchip DXP-STA*	10081627

** DXH-STA chips are available upon request. These DX chips utilize a carboxymethyl dextran matrix and follow the same protocol as polycarboxylate surfaces. However, for GCI, the recommended primary surfaces are PC chips, which feature a polycarboxylate matrix.*

Capture of biotinylated molecules to PCP- and PCH-RST KYMAchips

Overview

This protocol provides guidance for capturing biotinylated ligands on PCP- and PCH-RST KYMAchips, with the option for surface regeneration. The chip surface is functionalized with covalently bound single-stranded DNA (ssDNA). During the activation step, biotinylated ssDNA complexed with streptavidin (RST capture solution) is injected and anneals to the surface, forming double-stranded DNA (dsDNA). The bound streptavidin enables efficient capture of biotinylated ligands under physiological conditions without substantial changes in ionic strength or pH. Regeneration of the chip surface is achieved by denaturing the dsDNA by regeneration solution, preparing the surface for subsequent capture cycles.

In conjunction with the WAVE system, RST KYMAchips provide a convenient method for kinetic measurements of biotinylated ligands with various analytes.

Materials required

- WAVE RST kit:
  - PCP-RST or PCH-RST KYMAchip
  - RST capture solution 70x stock
  - Regeneration solution (50 mM NaOH, 1 M NaCl)
- Borate buffer (0.1 M borate, pH 9, 1 M NaCl)
- Running buffer
- Biotinylated ligand(s)

Notes

- We recommend aliquoting the 70x stock RST capture solution into small volumes (e.g., 10 µL) and storing the aliquots at -20 °C. Multiple freeze-thaw cycles are not recommended. Once diluted, the 1x capture solution is stable at 4 °C for up to 10 days.
- Desorb the instrument before starting an experiment using regenerable streptavidin KYMAchips.
- Let the chip equilibrate to room temperature before opening.
- The RST chips are not suitable for experiments involving DNA-binding proteins, as both the chip surface and the RST capture solution contain ssDNA.

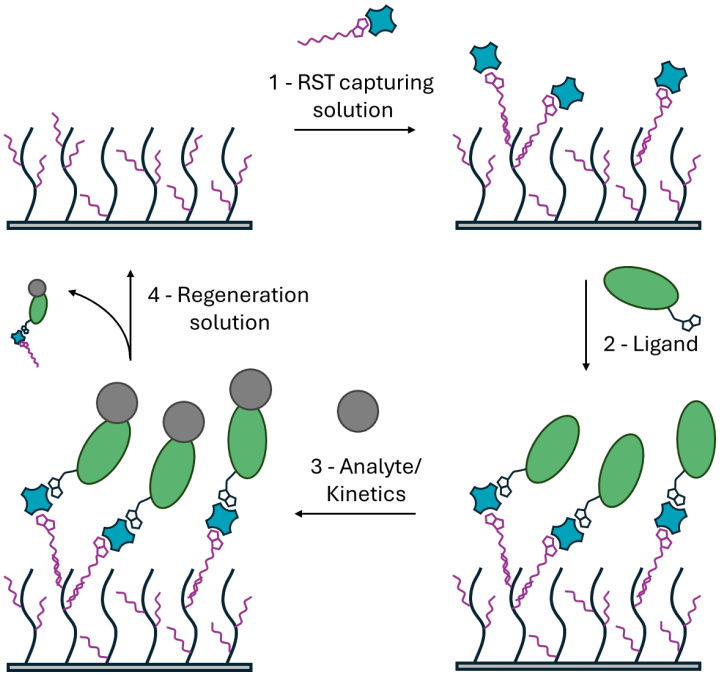


Figure 4. Schematic representation of the RST KYMAchip surface. Polycarboxylate matrix is pre-immobilized with ssDNA. 1: A complementary biotinylated ssDNA (purple) complexed with streptavidin (blue) is injected as the RST capture solution. 2: The surface is now prepared to capture biotinylated ligand (green) via the streptavidin. 3: The surface is then ready for molecular interactions analysis with the analyte (grey). 4: After the binding measurement the surface can be regenerated with the regeneration solution.

Procedure

Conditioning

Condition a KYMAchip by using the conditioning wizard in WAVEcontrol software. Select PCP-RST or PCH-RST as KYMAchip type. Standard conditioning cycles will be generated (*Table* 14).

Table 14. Standard conditioning cycles.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Conditioning	Borate buffer	10	180	60
2	Regeneration	1 M NaCl, 50 mM NaOH	80	30	60
3	Regeneration	1 M NaCl, 50 mM NaOH	80	30	60
4	Regeneration	1 M NaCl, 50 mM NaOH	80	30	60
5	Regeneration	1 M NaCl, 50 mM NaOH	80	30	60
6	Regeneration	1 M NaCl, 50 mM NaOH	80	30	60
7	Startup	Running buffer	10	60	60
8	Startup	Running buffer	10	60	60
9	Startup	Running buffer	10	60	60

Capture

Use the immobilization wizard to capture the ligand. Select “Capturing biotinylated ligands to regenerable streptavidin surface (RST)”. Select channel(s) to be activated (Flow path) and add information about the ligand. A series of cycles will be created (*Table 15*).

Table 15. Standard cycles for capture on RST KYMAchips. Parameters can be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Startup	Running buffer	10	60	60
2	Startup	Running buffer	10	60	60
3	Activation	RST capture solution	5	400	120
4	Capture	Ligand	5	420	60
5	Startup	Running buffer	10	60	60

Notes for Capture

- Startup cycles equilibrate the surface and allow the response to stabilize before the next cycle.
- The supplied RST capture solution is a 70× stock and must be diluted to 1x in the running buffer prior to use. Activation using the 1× RST capture solution facilitates the capture of biotinylated ligands. The default injection time is 420 seconds, though this can be adjusted as needed. Typical capture levels for the RST capture solution exceed 2000 pg/mm^2 for PCH-RST KYMAchips and 1000 pg/mm^2 for PCP-RST KYMAchips. The reference channel should also be activated with RST capture solution. Do not use an unmodified surface as reference.
- Capture is done by injecting the biotinylated ligand over the activated surface. Default contact time is 420 s but can be adjusted accordingly.
- When using a negative control protein on the reference channel, activate the channel and capture the biotinylated reference protein using the same procedure as for the ligand of interest.
- For initial binding assessment, we recommend using immobilization levels that yield a theoretical R_{max} between 20 and 100 pg/mm^2. For optimized data quality, consider lowering the level to reach R_{max} between 5 and 50 pg/mm^2.
- Ligand capture level can be controlled by using the Target Level function.
- Ligands should be prepared in the running buffer. Typical ligand concentrations are between 10 and 50 µg/mL. Lower concentrations can be used when low capture levels are required.

Kinetics

- Use one of the available wizards to run kinetics (e.g. RAPID Kinetics or Kinetics). The settings of the kinetics experiment should be based on the expected affinity of the interaction.
- It is recommended to run a dilution series of at least five concentrations of the analyte (0.1 x K_D to 10 x K_D) for traditional multicycle kinetics, or one concentration (10 x K_D or higher) for waveRAPID series. For more information refer to the *Kinetics Guide Section 3.5*.

Tips for optimal performance & troubleshooting

If the response after capture is not stable:

- Include multiple startup cycles before the kinetic assay to stabilize the surface.
- Try reducing the amount of captured ligand.

Regeneration

- For RST chips, the ligand screening approach allows different ligands to be tested for binding to a single analyte. Capture, regeneration, and kinetic parameters are configured using the Ligand Screening Wizard.
- Regeneration and re-capture protocols can also be implemented within other wizards.
- Various regeneration solutions can be used (*Table 16*). The standard ready-to-use solution (50 mM NaOH, 1 M NaCl) is generally sufficient for AVI-tagged proteins, ligands with a single biotin moiety (e.g., biotin-DNA), or chemically biotinylated proteins with low biotin density. Ligands with multiple biotin groups may require harsher regeneration conditions.
- If two regeneration cycles do not yield satisfactory results, additional regeneration injections may be necessary. After completing regeneration cycles, an extra injection with running buffer using the same settings is recommended to ensure stability.
- RST chips can typically maintain performance through 70 or more regeneration-recapturing cycles.
- Phase losses (signal jumps) may occur when injecting certain regeneration solutions (as noted in *Kinetics Guide section 4.1.1*). These fluctuations do not affect data integrity. If such signal shifts occur, the most reliable way to assess regeneration efficiency is to verify whether the RST capture solution level matches its pre-regeneration value.

Table 16. Regeneration solutions.

Regeneration solution	Injection duriation
50 mM NaOH 1M NaCl (standard)	2-4 x 30 s
30% acetonitrile 250 mM NaOH *	2-4 x 30 s
6 M guanidine 250 mM NaOH *	2 x 30 s

** For bigger/highly biotinylated ligands. Solutions need to be prepared fresh. Might show phase jumps.*

Additional information

For more details, please visit our *Knowledge Center* (https://www.malvernpanalytical.com/en/learn/knowledge-center) or consult the *Kinetics Guide – Binding Kinetics with the WAVEsystem*.

The guide can be also found in WAVEcontrol under the Help tab.

Table 17: Ordering information

Consumable	Product number
KYMAchip PCP-RST kit	10087543
KYMAchip PCH-RST kit	10087768
RST – Capture Solution for 50 Cycles	10057702

Capture of antibodies or Fc-tagged proteins to PCP-PAG KYMAchips

Overview

This protocol gives recommendations for capturing antibodies or Fc-tagged proteins (ligands) to PCP-PAG KYMAchips. The linear polycarboxylate matrix on the KYMAchip surface is functionalized with covalently attached fusion protein A/G. This enables site-directed ligand capture through the interaction of Fc region of antibody (or Fc tag) with protein A/G in physiological conditions.

The PCP-PAG KYMAchip sensor surface can be regenerated using a low pH glycine solution or phosphoric acid.

In conjunction with the WAVEsystem, PCP-PAG KYMAchips provide a convenient method for performing kinetic measurements of antibodies with various analytes.

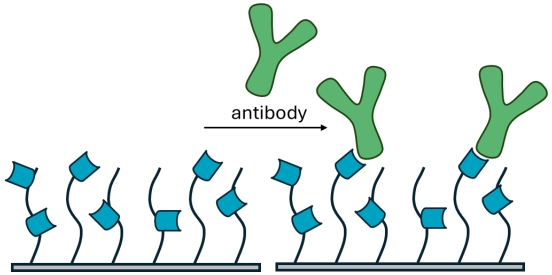


Figure 5. Schematic representation of PCP-PAG KYMAchip surface. Polycarboxylate matrix is pre-immobilized with protein A/G (blue). The antibody (green) is captured by protein A/G via the Fc region. The surface is then ready for molecular interaction analysis.

Materials required

- PCP-PAG KYMAchip
- Borate buffer (0.1 M borate, pH 9, 1 M NaCl)
- Running buffer
- Ligand (antibody or Fc-tagged protein)

Notes

- Desorb the instrument before starting the experiment with PCP-PAG KYMAchips.
- Make sure to let the chip equilibrate to room temperature before opening.

Procedure

Conditioning

Condition a KYMAchip by using the conditioning wizard in WAVEcontrol software. Select PCP-PAG as KYMAchip type. Standard conditioning cycles will be generated (*Table* 18).

Table 18. Standard conditioning cycles.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Conditioning	Borate buffer	10	180	60
2	Startup	Running buffer	10	60	60
3	Startup	Running buffer	10	60	60
4	Startup	Running buffer	10	60	60

Capture

Use the immobilization wizard to capture ligand. Select “Capturing of antibody to protein A/G surface” and fill in the information about the ligand(s). A series of cycles will be created (*Table* 19).

Table 19. Standard cycles for antibody capture. Parameters can be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Startup	Running buffer	10	60	60
2	Startup	Running buffer	10	60	60
3	Capture	Ligand	10	420	60
4	Startup	Running buffer	10	60	60

Notes for capture

- Startup cycles equilibrate the surface and allow the response to stabilize before the next cycle.
- Capture is done by injecting the ligand solution over the surface. Default contact time is 420 s but can be adjusted accordingly.
- For initial binding assessment, we recommend using capture levels that yield a theoretical R_{max} between 20 and 100 pg/mm^2. For optimized data quality, consider lowering the immobilization level to reach an R_{max} between 5 and 50 pg/mm^2.
- Ligand capture level can be controlled by using the Target Level function.
- The ligand should be prepared in the running buffer. Typical ligand concentrations range between 500 ng/mL and 20 µg/mL.

Kinetics

- Use one of the available wizards to run a kinetics assay (e.g. RAPID Kinetics or Kinetics). The settings of the kinetics experiment should be based on the expected affinity of the interaction.
- It is recommended to run a dilution series of at least five concentrations of the analyte (0.1 x K_D to 10 x K_D) for traditional multicycle kinetics or one concentration (10 x K_D or higher) for a waveRAPID series. For more information refer to the *Kinetics Guide Section 3.5*.

Tips for optimal performance & troubleshooting

If the response after ligand capture is unstable:

- Include multiple startup cycles before the kinetic assay to stabilize the surface.
- Consider reducing the amount of captured ligand.

Regeneration

- Regeneration can be performed during ligand screening or when working with high-affinity interactions. It is typically included as part of the assay and can be configured using software wizards (e.g., ligand screening, kinetics, etc.). For PCP-PAG KYMAchips, regeneration removes the entire ligand-analyte complex (i.e., the ligand captured to the protein A/G and the bound analyte) from the surface, and ligand re-capture is required before the next kinetic cycle.
- Regenerate the surface by injecting a regeneration solution for 30 s with 120 s rinse. Typical regeneration solutions include 10 mM glycine at pH 1.5 or 100 mM phosphoric acid (H_3PO_4).
- If a single regeneration cycle is insufficient, additional regeneration injections should be included.

Additional information

For more details, please visit our *Knowledge Center* (https://www.malvernpanalytical.com/en/learn/knowledge-center) or consult the *Kinetics Guide – Binding Kinetics with the WAVEsystem*.

The guide can be also found in WAVEcontrol under the Help tab.

Table 20: Ordering information

Consumable	Product number
KYMAchip PCP-PAG	10081595

Capture of lipid membrane vesicles to PCP-LIP KYMAchips

Overview

This protocol gives recommendations for capturing lipid vesicles with or without membrane proteins to PCP-LIP KYMAchips. The chip surface is functionalized with lipophilic alkyl chains that act as anchors for lipid membrane-based particles such as proteoliposomes, nanodiscs, reconstituted membranes, virus-like lipoparticles (enveloped VLPs), and other lipoprotein particles.

The sensor surface can be regenerated using different solutions that disrupt the non-covalent hydrophobic interaction of the ligand on the surface.

In conjunction with the WAVEsystem, PCP-LIP KYMAchips provide a convenient method for kinetic measurements of lipid membrane vesicles with various analytes.

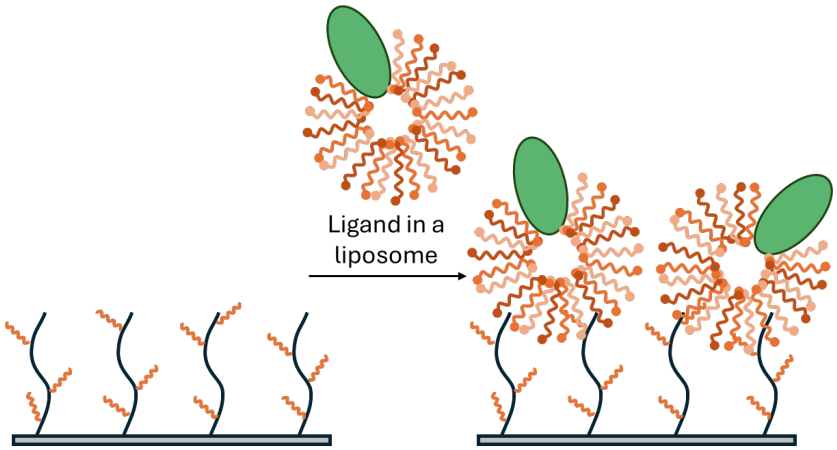


Figure 6. Schematic representation of PCP-LIP KYMAchip surface. A polycarboxylate matrix is pre-immobilized with lipophilic alkyl chains (orange). The lipid membrane vesicle containing the ligand (green) binds non-covalently to the lipophilic surface. The surface is then ready for molecular interactions analysis.

Materials required

- PCP-LIP KYMAchip
- Borate buffer (0.1 M borate, pH 9, 1 M NaCl)
- 20 mM CHAPS
- Detergent-free running buffer
- Ligand

Procedure

Conditioning

Condition a KYMAchip by using the conditioning wizard in WAVEcontrol software. Select PCP-LIP KYMAchip type. Standard conditioning cycles will be generated (*Table 1*).

Table 21. Standard conditioning cycles.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Conditioning	Borate buffer	30	180	60
2	Conditioning	20 mM CHAPS	10	60	60
3	Conditioning	20 mM CHAPS	10	60	60
4	Conditioning	20 mM CHAPS	10	60	60
5	Startup	Running buffer	30	60	60
6	Startup	Running buffer	30	60	60
7	Startup	Running buffer	30	60	60

Capture

Use the immobilization wizard to capture ligand. As capture works by injecting the ligand solution over the sensor surface (the same as for some of our other chip types), select "Capturing of biotinylated ligand to streptavidin surface". Add information about the ligand and the series of cycles will be created (*Table 22*).

Table 22. Standard cycles for hydrophobic capture. Parameters can be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Startup	Running buffer	10	60	60
2	Startup	Running buffer	10	60	60
3	Capture	Ligand	10	420	60
4	Startup	Running buffer	10	60	60

Notes for capture

- Startups equilibrate the surface and allow the response to stabilize before the next cycle.
- Capture is done by injecting a ligand solution over the surface. Default contact time is 420 s but can be adjusted accordingly. Longer injections may be needed to obtain high capture levels.
- (Proteo-)liposomes or other lipoprotein particles should be prepared in running buffer containing around 0.5 – 2 mM lipid concentration.
- When working with lipid vesicles/nanodiscs with membrane proteins, reference channel can be loaded with empty vesicles/nanodiscs.
- For initial binding assessment, we recommend using immobilization levels that yield a theoretical R_{max} between 20 and 100 pg/mm^2. For optimized data quality, consider lowering the level to reach R_{max} between 5 and 50 pg/mm^2.
- Ligand capture level is controlled by varying ligand concentration, injection time and/or number of capture cycles.
- Additional washing steps of 10 – 100 mM NaOH at higher flowrates can remove loosely bound vesicles. Check the vesicle compatibility for this step.
- The running buffer should not contain any detergents.

Kinetics

- Use one of the available wizards to run kinetics (e.g. RAPID Kinetics or Kinetics). The settings of the kinetics experiment should be based on the expected affinity of the interaction.
- It is recommended to run a dilution series of at least 5 concentrations of the analyte (0.1 x K_D to 10 x K_D) for traditional multicycle kinetics or one concentration (10 x K_D or higher) for waveRAPID series. For more information refer to the *Kinetics Guide Section 3.5*.

Tips for optimal performance & troubleshooting

If the response after capture is not stable:

- Include multiple startup cycles before the kinetic assay to stabilize the surface.
- Try reducing the amount of captured ligand.
- Ensure that the running buffer does not contain any detergent.
- If a detergent-containing running buffer was previously used, desorb or sanitize the device before conducting PCP-LIP experiments.

Regeneration

- Regeneration may be performed (e.g., for ligand screening or when working with tight binders) by stripping vesicles from the surface via injection of a regeneration solution (*Table 23*). Most common regeneration solutions are detergents (e.g., 20 mM CHAPS) and isopropanol mixtures. Ligand re-capture must be completed prior to the next kinetic cycle. Regeneration and re-capture steps are typically integrated into the assay and can be configured using software wizards (e.g., for ligand screening or kinetic analyses).
- Regeneration scouting might be needed to find the best regeneration solution.
- The removal of anchored ligand(s) may require several regeneration injections and/or subsequent injections of different regeneration solutions listed in *Table 23*.

Table 23. Regeneration solutions.

Regeneration solution	Injection duration
Isopropanol : 50 mM NaOH, 2:3 (v/v)	30 s – 3 min injection
Isopropanol : 100 mM NaCl, 1:1 (v/v)	30 s – 3 min injection
0.5% SDS	30 s – 3 min injection
20 mM CHAPS	30 s – 3 min injection
40 mM Octylglucopyranoside	30 s – 3 min injection
other detergent solutions	30 s – 3 min injection

Additional information

For more details, please visit our *Knowledge Center* (https://www.malvernpanalytical.com/en/learn/knowledge-center) or consult the *Kinetics Guide – Binding Kinetics with the WAVEsystem*.

The guide can be also found in WAVEcontrol under Help tab.

Table 24: Ordering information

Consumable	Product number
KYMAchip PCP-LIP	10081620

Amine-coupling of molecules to PCZ KYMAchips

Overview

This protocol provides recommendations for immobilizing negatively charged or low-pI ligands onto PCZ KYMAchips via amide bond formation between primary amines on the ligand and carboxyl groups on the chip surface. The PCZ surface is a zwitterionic derivative of the linear polycarboxylate. It contains both negatively charged carboxyl groups and positively charged tertiary amines in similar concentrations, which facilitates immobilization of negatively charged ligands.

The PCZ matrix on the KYMAchip surface is first activated with a mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to create reactive succinimide esters neutralizing the negatively charged carboxyl groups. This step temporarily alters the surface charge to a net positive, facilitating the preconcentration and subsequent coupling of the negatively charged ligand. The ligand is prepared in a low salt buffer lacking primary amines at a pH above its isoelectric point (pI), ensuring it remains negatively charged. After coupling and quenching of the active NHS esters, the net charge becomes neutral again and the surface is ready for interaction analysis.

Amine coupling creates a permanent covalent attachment between the ligand and the chip surface, which means that the ligands cannot be removed in the regeneration step.

In conjunction with the WAVEsystem, PCZ KYMAchips provide a convenient method for kinetic measurements of untagged ligands with low pI with various analytes.

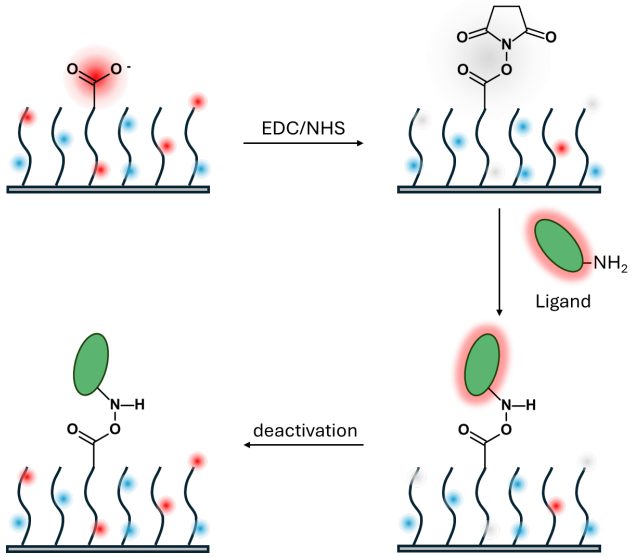


Figure 7. Schematic representation of the PCZ KYMAchip surface with a zwitterionic surface matrix containing a similar amount of positive (blue) and negative (red) charges, resulting in a neutral net surface charge. The surface is first activated with a mixture of NHS and EDC, which reacts with negatively charged carboxyl groups to form uncharged NHS esters (grey). This activation step temporarily shifts the surface charge to net positive, enabling reverse-charge preconcentration and immobilization of the negatively charged ligand (depicted in green with a red glow). This is followed by a deactivation/passivation step, restoring a net neutral surface charge.

Materials required

- PCZ KYMAchip
- Borate buffer (0.1 M borate, pH 9, 1 M NaCl)
- 0.2 x running buffer
- Ligand
- Ligand buffer (1-1.5 pH units above ligand pI and low ionic strength, e.g. 10 mM MES pH 6.5)
- Activation reagent
  - 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)
  - 100 mM N-hydroxysuccinimide (NHS) in 50 mM MES pH 5.0
- Passivation reagent
  - Running buffer at physiological pH

Process

Conditioning

Condition a KYMAchip by using the conditioning wizard for PCZ. Standard conditioning cycles will be generated *Table* 25).

Table 25. Standard conditioning cycles.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Conditioning	Borate buffer	10	180	60
2	Startup	0.2x Running buffer	10	60	60
3	Startup	0.2x Running buffer	10	60	60
4	Startup	0.2x Running buffer	10	60	60

Immobilization

Use the immobilization wizard to capture ligand. Select "Amine coupling to carboxymethyl surface", channel(s) to be activated (Flow path) and add information about the ligand. Deselect passivation in the immobilization series. Passivation will be set up as a separate series. The series of cycles will be created (*Table* 26*Table* 2).

Table 26. Standard cycles for amine coupling. Parameters can be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Activation	EDC/NHS	10	420	60
2	Immobilization	0.2x Running buffer	10	420	60

Passivation

For deactivation of unreacted esters and to bring the surface charge back to neutral, the remaining NHS esters are hydrolysed at physiological pH for ~1 h.

Set up a separate passivation series consisting of 15 startups using a physiological pH buffer. To do this, create 15 startups using the kinetics wizard without adding analyte. Switch to a physiological buffer of your choice and perform a buffer exchange to remove the previous buffer from the flow paths before starting the series.

Table 27. Standard cycles for passivation. Parameters can be adjusted.

Cycle	Type	Sample	Flow rate (µl/min)	Injection (s)	Rinse (s)
1	Startup	Running Buffer pH 7	60	60	60
...	Startup	Running Buffer pH 7	60	60	60
15	Startup	Running Buffer pH 7	60	60	60

Notes for immobilization and passivation

- Activation with EDC/NHS is done to activate carboxylic groups for amine coupling. Use 100 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (in water) and 100 mM N-hydroxysuccinimide (NHS) (in 50 mM MES buffer, pH 5). Mix 1 :1 (v/v) just before starting the series.
- Note that the half-life of the reactive succinimide ester is in the order of minutes at pH 8-9 up to a few hours at pH 4-5 in aqueous buffers.
- Immobilization is done by injecting ligand solution over the activated surface. Default contact time is 420 s but this can be adjusted accordingly.
- Ligand capture level is controlled by varying ligand concentration, injection time and/or number of capture cycles. We suggest keeping immobilization levels as low as possible.

- Ligand should be prepared in the appropriate buffer, 1-1.5 pH unit above pI, e.g. 10 mM MES pH 6.5. Concentrations between 10 and 50 µg/ml are typical.
- Passivation series should take around 1 h with running buffer at physiological pH to allow the remaining NHS to hydrolyze. Alternatively, moderate alkaline buffer (pH 8.0) can be injected for a few minutes.

Kinetics

- Switch to a physiological buffer of your choice (if not already performed for the passivation series) and perform a buffer exchange to remove the previous buffer from the flow paths. Begin the interaction measurements.
- Use one of the available wizards to run kinetics (e.g. RAPID Kinetics or Kinetics). The settings of the kinetics experiment should be based on the expected affinity of the interaction.
- It is recommended to run a dilution series of at least five concentrations of the analyte (0.1 x K_D to 10 x K_D) for traditional multicycle kinetics, or one concentration (10 x K_D or higher) for waveRAPID series. For more information refer to the *Kinetics Guide Section 3.5*.

Tips for optimal performance

- Always prepare EDC/NHS mixture immediately before starting the immobilization series.
- Quality of immobilization reagents is crucial for amine coupling to be successful. Use only high-quality reagents dedicated to ligand immobilization.
- Proteins can exhibit limited stability when exposed to low-ionic-strength used for preconcentration. To minimize protein exposure to undesirable conditions, dilute the ligand in coupling buffer prior to use.
- Amine coupling immobilizes proteins in a random orientation, rendering some binding sites inaccessible to the analyte. Take this into consideration when calculating ligand density.

Troubleshooting

If ligand immobilization levels are low, check the measurements and identify the cause of low ligand levels.

If preconcentration is not satisfactory (little increase of response during ligand injection):

- Make sure that the ionic strength of the ligand buffer is low (ligand is sufficiently diluted or desalted).
- Increase the concentration of the ligand and/or contact time.
- Check the shape of EDC/NHS injection. If the signal increases significantly during the second part of the injection, consider reducing the EDC concentration or switching to an alternative EDC.
- Note: due to the change in charges during the activation step, pH scouting cannot be used to optimize preconcentration conditions.

If the ligand is preconcentrated on the surface (high response during injection) but not immobilized (low level in baseline after injection):

- Make sure to use fresh activation reagents.
- Ensure that buffers do not contain primary amines (e.g., Tris) or sodium azide.
- Consider different immobilization/capture method.

If the immobilization level seems sufficient, but analyte responses are too low:

- Increase the immobilization level of the ligand on the surface. Amine-coupled ligands typically exhibit lower binding activity than captured ligands.
- Check the stability of protein in the coupling buffer.
- Consider different immobilization/capture methods.

Regeneration

When working with tight-binding interactions, regeneration of the KYMAchip surface is necessary. Since amine-coupled ligands are irreversibly bound to the surface only the analyte-ligand interaction can be disrupted. It is recommended to perform scouting experiments to find optimal regeneration conditions. Common procedures for regeneration of the ligand surface consist of short injection pulses of solutions with extreme pH levels, high salt concentration or interaction-specific reagents (*Table* 28).

Table 28. Regeneration solutions.

Interaction type	Regeneration solution	Injection duration
Protein (e.g. anti-IgG or protein A)	10 mM Glycine pH 1.5-2.5 or 100 mM H_3PO_4	One or more injections of 30 s
ssDNA-ssDNA	6-7 M Urea or 100 mM NaOH	30 s injection *
Hydrophobic	0.2 M CHAPS or other detergent	3 min injection
Charge driven	0.5-1 M NaCl or high/ low pH buffers	30 s injection *
Displacement (small molecule – enzyme)	High conc. competitor (low affinity)	30 s - 3 min injection *

** Phase jumps can occur during regeneration*

We suggest keeping the conditions as mild as possible to not damage the ligand. To reduce exposure to the regeneration solution, the solutions should be injected for a short duration, but this process can be repeated to ensure full regeneration of the ligand. For more information refer to the *Kinetics Guide Section 3.1.4*.

Additional information

For more details, please visit our *Knowledge Center* (https://www.malvernpanalytical.com/en/learn/knowledge-center) or consult the *Kinetics Guide – Binding Kinetics with the WAVEsystem*.

The guide can be also found in WAVEcontrol under Help tab.

Table 29: Ordering information

Consumable	Product number
KYMAchip PCZ	10081599

Order information

Consumable	Product number
KYMAchip PCP	10081626
KYMAchip PCH	10081623
KYMAchip PCL	10081592
KYMAchip PCP-NTA	10081628
KYMAchip PCH-NTA	10081624
KYMAchip PCP-STA	10081596
KYMAchip PCH-STA	10081621
KYMAchip PCP-RST kit	10087543
KYMAchip PCH-RST kit	10087768
KYMAchip PCP-PAG	10081595
KYMAchip PCP-LIP	10081620
KYMAchip PCZ	10081599
KYMAchip DXP	10081625
KYMAchip DXP-STA	10081627
KYMAchip DXH	10081593
RST – Capture Solution for 50 Cycles	10057702

Malvern
Panalytical